STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN CATTLE AND SHEEP.

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Introduction.

The study of the physiology of reproduction in domesticated animals is of primary importance for the elucidation of the many and diverse problems of fertility and sterility. Without this basic knowledge, reproductive disorders are improperly understood and any attempt at improvement in breeding performance and in treatment of sterility is merely empirical.

Our knowledge of reproductive functions has increased considerably within recent years but much still remains to be done, particularly on the manner in which environmental conditions influence reproduction in endogenous and exogenous animals. The study of reproductive functions under varying environmental conditions is important, for the influence of a particular environmental factor may not be evident under all conditions. Since, also, reproductive functions are influenced by varying environmental conditions they may serve as an index of the relations between an animal and its environment.

The work described in the following series of papers was undertaken primilarly to study the physiological aspects of reproduction in cattle and sheep under the varying environmental conditions in Kenya, the climate of which is that of a tropical plateau. It falls into four sections:- (1) the periodicity and duration of oestrus in cattle, (2) ovulation and the hormonal induction of oestrus in the ewe; (3) the semen of the bull, and (4) artificial insemination. From time to time certain aspects

of this work necessarily received greater attention than others, as, for example, the development and application of artificial insemination to combat coital disease in cattle, and as a direct means of studying reproduction in both cattle and sheep, for which it has proved a valuable tool. Much work on problems of male and female reproduction has therefore been stimulated by the practice of artificial insemination. Its use, for example, has led to the evolution of standards, hitherto lacking, for the assessment of male fertility and to the more intensive study of sexual periodicity and the factors which influence it.

It is generally recognised that the majority of animals breed at a certain season or seasons of the year. The whole subject of sexual periodicity and the causes which determine it have been fully reviewed by Marshall (1936, 1937, 1942). Marshall concluded that in all mammals and probably in all vertebrates, there is an internal sexual rhythm, with alternating periods of rest and activity. Usually the cycle is adjusted to external seasonal changes and the period of greatest sexual activity is usually, though not invariably, in the spring, but there is much specific variation and among ruminating mammals, breeding is general in autumn. In tropical and sub-tropical animals breeding is not nearly so restricted to definite seasons and it appears that ecological factors play a part in its recurrence. There may be two seasons of sexual activity in the year in individual animals transported across the equator and the times of breeding in such animals are eventually reversed. With the majority of vertebrates, exteroceptive stimuli, dependent on light

and ultra-violet irradiation are of primary importance in adjusting the cycle to changing periodic environmental conditions. The factors in question act through the intermediation of the central nervous system and the anterior pituitary.

The proximate causes of a breeding seasons are of two quite different kinds (Baker, 1938). There are (1) external environmental factors such as light, temperature and rainfall and (2) internal or inherent factors. There is ample evidence that external factors play a part in regulating reproductive functions in animals and birds. Internal rhythm may act in many species by making them responsive to external factors, yet, according to Baker and Baker (1936), it can never wholly account for the timing of the breeding seasons, for it would get quite out of step with the sun in the course of the ages.

In animals which breed throughout the year, evidence of an optimum season for mating is more difficult to get. Hammond (1925) however, has shown that while rabbits are capable of breeding throughout the year they will copulate more readily in the spring and summer months from April to July, than they will in the autumn and winter months from September to March. Hammond (1927) also noted seasonal variation in sexual periodicity in the cow in Britain. It appeared that under the totally different environmental conditions in Kenya, cattle might tend to breed more particularly at a certain period or periods of the year. Evidence supporting this view and an analysis of the climatic factors which may be involved, are presented.

The Merino ewe experiences a marked seasonal variation in reproductive capacity, as shown by the length of the oestrous cycle and the incidence of oestrus. With the object of overcoming adverse

seasonal influences which lower fertility, it appeared important to establish some form of artificial hormonal control of the oestrous cycle in the ewe.

The accurate determination of the time of ovulation in the ewe is of practical importance in ascertaining the best time for mating. The observations recorded later, were accordingly undertaken to assist in determining the optimum time for the introduction of sperm into the female genital tract in artificial insemination experiments.

Measurements on the semen of animals can be divided broadly into two classes, (a) quantitative measurements, such as the estimation of number of spermatozoa per unit volume and (b) those which are qualitative, or at best semi-quantitative, such as the estimation of activity. Determination of activity may, however, be placed, indirectly, on a quantitative basis by its high correlation with the initial pH of the semen and the pH change on incubation. In general, using all available criteria, it is possible to identify a characteristic type of semen with fertility, sterility or with "reduced" fertility. Present work would seem to indicate that most promise of advance in this direction lies in the study of the metabolism of spermatozoa.

The successful application of artificial insemination depends on sound basic knowledge of reproductive functions. Because of variation in fertility caused by environmental conditions, it is essential that problems of reproduction should be studied wherever its application is practised. In the U.S.A. in States in which artificial insemination is being developed on sound lines, there is very close co-operation between the Artificial Insemination

Societies and the State University, in which research on current problems is conducted.

Artificial insemination can provide a means of improving the cattle in the British Empire, which probably cannot be achieved in any other way. The magnititude of the task, which is the genetic improvement of the cattle, is indicated by the size of the cattle population, which is stated to be about 20 million (Duckham, 1932).

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STUDIES ON REPRODUCTION IN CATTLE

PT. I. THE PERIODICITY AND DURATION OF OESTRUS

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IT has been observed that Zebu cattle in East Africa appear to exhibit a seasonal fertility, in that fertile matings tend to occur more particularly at a certain season of the year (unpublished results). It seemed that this apparent seasonal fertility could best be explained by assuming that conditions for the occurrence of oestrus and ovulation were more favourable at this time of the year than at any other. Considerable variation in the duration and intensity of oestrus is known to occur, and Hammond [1], working under experimental conditions, noted that in Britain the average duration of oestrus is longest in the summer months and shortest in the winter months.

Observations were accordingly undertaken, first, to ascertain if significant variation in the duration of oestrus in Zebu cattle did occur from season to season, and secondly, to investigate the relationship of any such variation in the duration of oestrus to seasonal variation in environmental conditions.

Experimental Material and Methods

In order that all seasonal changes be noted, as complete a record as possible was kept of environmental conditions, coincident with the observations on the oestrous cycle. Meteorological records—rainfall, maximum and minimum temperatures, and, as no sunshine-recording apparatus was available, observations on the amount of cloud—were made daily. Monthly analysis of the pasture was very kindly undertaken by Prof. R. G. Linton.

All the observations were made on a stock-farm in North Kavirondo in Kenya Colony. The farm is situated approximately 50 miles north of the equator (0·31° N. and 34·30° E.) at an altitude of about 4,900 ft. above sea-level. Normally there are two rainy seasons in the year: the long rains occur approximately from March to June, and the short rains in August, September, and October. The average annual rainfall for this district, recorded at two stations for 11 and 8 years, respectively, is 68·41 in. The wettest months of the year are March (av. 6·11 in.), April (av. 9·15 in.), May (av. 9·58 in.), June (av. 6·02 in.), August (av. 8·96 in.), and September (av. 6·37 in.). July with an average rainfall of 5·94 in. is the driest month between the long and short rains. January with an average of 2·26 in. is the driest month of the year, closely followed by November, December, and February. No temperature records are available for this district as a whole, but those from an adjoining district (recorded at a station approximately 50 miles from the above stock-farm and at a slightly higher altitude) show that the maximum and minimum temperature range for the year is comparatively

slight. The mean of maximum and minimum temperatures for 1931 was 73·4° F. and 50·3° F., respectively; the absolute maximum temperature was 83·0° F., and the absolute minimum temperature was 43·1° F.

Originally 11 animals were chosen for the experiment, but for various reasons not connected with the experiment 6 were discarded. Of the 5 animals that were used for the observations on the oestrous cycle, 2 were cows and 3 were heifers. The cows had each borne one calf, but neither was milking during any period of the time they were under observation. The average ages of the cows and heifers were $4\frac{1}{2}$ and 3 years, respectively. All 5 animals gave a negative reaction to the contagious-abortion test at the beginning of the experiment.

The experiment proper began in March 1933. It had been intended to regard the period March–April as a preliminary period and to begin the experiment in May, but unforeseen circumstances necessitated finishing the experiment in February 1934; experimental details are, however, given from March in order that a complete year may be covered. There are, moreover, no reasons why data from the period March–April should not be considered with the rest of the data. From March until the end of April two vasectomized bulls were used. The procedure adopted at this time was as follows: after a bull had served a cow he was removed from the paddock and immediately replaced by the other bull. This bull was removed in 30 min. or on service, whichever was the lesser period, and the other bull was returned. This procedure was continued until the end of oestrus. On a few occasions when a further service had not taken place in 30 min., the bull was left with the cow until service occurred, or indefinitely when no service took place. It was noted that on three occasions bull No. 22 failed to serve an oestrous cow although with her for 30 min., and on another occasion bull No. 21 did not serve an oestrous cow although with her for well over an hour. As the duration of oestrus seemed to be very short it was decided to use only one bull for further observations, and from May onwards bull No. 21 was used, except for a few occasions when two cows coming on 'heat' at about the same time necessitated the use of both bulls, and in November when bull No. 21 was suffering from phimosis and was unfit for service. As both bulls had several times been observed to take a long time to serve a cow on 'heat' the following procedure was adopted and used from May until the end of the observations in the following February. The 5 experimental animals were kept with the vasectomized bull in two paddocks, each of which was approximately four acres in extent. The animals were moved from one paddock to the other according to the state of the grazing. Owing to the scarcity of grazing during the dry season (December, January, and February) the animals were allowed to graze outside, but in the neighbourhood of the paddocks. Once a day the animals were watered at a stream half a mile away. Since the bull was with the cows all the time, a cow was noticed immediately she began to show signs of coming on 'heat'. If this occurred during the day the oestrous cow was allowed to remain with the rest of the experimental animals, but if at night, the cow was placed in a small paddock adjoining the larger ones for ease of observa-

tion. The approximate date on which a cow was due to come on 'heat' was known from previous records. If a cow had not come on heat by the seventeenth day after the beginning of the previous oestrous period she was placed in the small paddock that night and kept under observation night and day until she did. The exact time of the initiation of oestrus was thus known.

The bull was removed from the cow immediately after service had taken place. After half an hour he was put back with the cow and left with her, either until a further service took place, in which case he was again removed for half an hour, or indefinitely when no service occurred. In this way there was no possibility of service not taking place through the bull being with the cow for too short a time. The greatest number of services performed by bull No. 21 within a short period—5 in 6 hours and 8 in 28 hours—took place when 3 cows came on 'heat' within a day of each other. This number of services appeared to have no adverse effect on the sexual capabilities of the bull. Throughout the experiment bull No. 21 performed 124 services and bull No. 22 27 services. Oestrus was recognized solely by the occurrence of mating, and the duration of oestrus was estimated as the time between the first and last service in the one oestrous period. External signs of oestrus were usually so slight as to be unrecognizable. Occasionally a flow of mucus, at first clear and fairly fluid, later thicker and whitish, occurred at the time of oestrus. An animal coming on 'heat' was easily recognized by the bull following her about, standing near her, and from time to time, particularly as the onset of oestrus approached, attempting to mount her. The duration of the dioestrous cycle was taken as the interval between the beginning of one oestrus and the beginning of the subsequent oestrus.

The Periodicity of Oestrus

It is usually considered that the dioestrous cycle in cattle lasts for approximately 21 days. Hammond, who investigated 58 cycles, using a vasectomized bull, found the range of variation to be from 16·6 to 24·0 days; the mean was 19·2 days and the mode 17·8 days. Frei and Metzger [2] give a slightly greater range of variation, namely, 15 to 25 days. In their investigations the mean was 20·2 days and the mode 19·1 days.

Particulars of the duration of the dioestrous cycle, noted by different workers, are summarized in Table 1. A range of variation exceeding that of 15–25 days may possibly in some instances be due to individual differences, but very extreme variation in the length of the cycle is undoubtedly due to the inclusion of abnormal cycles. It will be seen from Table 1 that the mean length of the cycle, as observed by different workers, lies between 19 and 21 days, and the mode in those cases in which it can be accurately determined between 17·8 and 20·8 days.

Sixty-three cycles in 5 animals were investigated by the author. Details of these cycles are given in Table 2. The range of variation in the duration of the cycle is from 17·9 to 24·1 days; the mean duration is 20·1 days and the mode 20·7 days. The distribution of the cycles of various durations is given in Fig. 1.

TABLE 1. *Duration of the Dioestrous Cycle*

Breed	*Country*	*No. of animals*	*No. of cycles*	*Range of variation (days)*	*Mode (days)*	*Mean (days)*	*Authority*
Shorthorn	Britain	15	58	16·6–24·0	17·8	19·2	Hammond (1927)
Brown	Germany	..	393	8·0–32·0	20·8	21·7	Wagner (1931)
	Germany	..	59	15·0–25·0	19·1	20·2	Frei and Metzger (1926)
	Britain	11	..	17·2–21·2	19·2	19·1	Marshall (1924)
Zebu	Kenya	5	63	17·9–24·1	20·7	20·1	Anderson
	Germany	38	350	6·0–30·0	18–22	..	Struve (1911)
	Germany	12	93	..	18–24	..	Schmid (1902)
	Germany	..	..	..	21	..	Kupfer (1920)
	Germany	..	..	..	21	..	Zeitschmann (1921)
	Germany	..	..	..	21–8	..	Schmaltz (1926)
	Sicily	..	..	..	22	..	Alongi (1924)
	Italy (Umbria)	..	..	..	22–3	..	,, ,,
	Italy (Umbria)	..	..	..	21–8	..	Sanctis (1926)
	Germany	..	..	17·5–28·0	..	..	Weber (1911)
	France	..	..	..	21	..	Curot (1921)
	Germany	..	..	..	21–8	..	Franck-Albrecht (1914)

TABLE 2. *Duration of the Dioestrous Cycle in hours Zebu Animals*

No. of animals	*1933*										*1934*	
	Mar.	*April*	*May*	*June*	*July*	*Aug.*	*Sept.*	*Oct.*	*Nov.*	*Dec.*	*Jan.*	*Feb.*
31	493	528	492	505	521	506	512 519	466	580	479 512	514	510
66		469 472	435		470 474	451	493 430	487 444	457	458 431	441	467
72		455 477			435 446	446 485	432 464	453 444	479	460	487 454	
80					514 506	514		493	494 457	548	542	490
90	464	497		492		473	548 531	479	484 494	549		
Av.	478·5	483	463·5	498·5	480·8	479·2	491·1	466·4	492·3	491	487·6	489
No. of cycles	2	6	2	2	7	6	8	7	7	8	5	3

The mean length of the cycle in Zebu cattle (20·1 days) is slightly greater than the figure given by Hammond for Shorthorn crossbreds in Britain (19·2 days), and the mode is also slightly greater. Both figures, however, are well within the range given for European cattle.

Hammond has noted that cows have a slightly longer oestrous cycle than heifers, the average difference being 11 hours. There is no difference in this respect in the animals examined by the author (Table 3).

TABLE 3. *Duration of the Dioestrous Cycle in Zebu Cows and Heifers*

	No. of animal	*No. of cycles*	*Duration of Cycle in Days*		
			Average	*Min.*	*Max.*
Heifers	72	14	18·8	18·0	20·3
	80	9	21·1	19·0	22·8
	90	10	20·9	19·3	22·8
	Average for heifers		20·3	18·8	21·9
Cows	31	14	21·3	19·4	20·0
	66	15	19·1	17·9	20·4
	Average for cows		20·2	18·7	20·2

The monthly variation in the duration of the cycle is shown in Table 2. The longest cycle—498·5 hours—occurs in June and the shortest in May. During the four months November, December, January, and February the cycle maintains a consistently high level, namely, between 488 and 492 hours.

Hammond found that on the average the cycle was about 40 hours longer in the summer than in the winter or spring. Wallace, on the other hand, states that the cycle is shorter in summer than in winter. Wagner [3] gives the length of the cycle in summer and winter as 22·3 and 22·6 days, respectively.

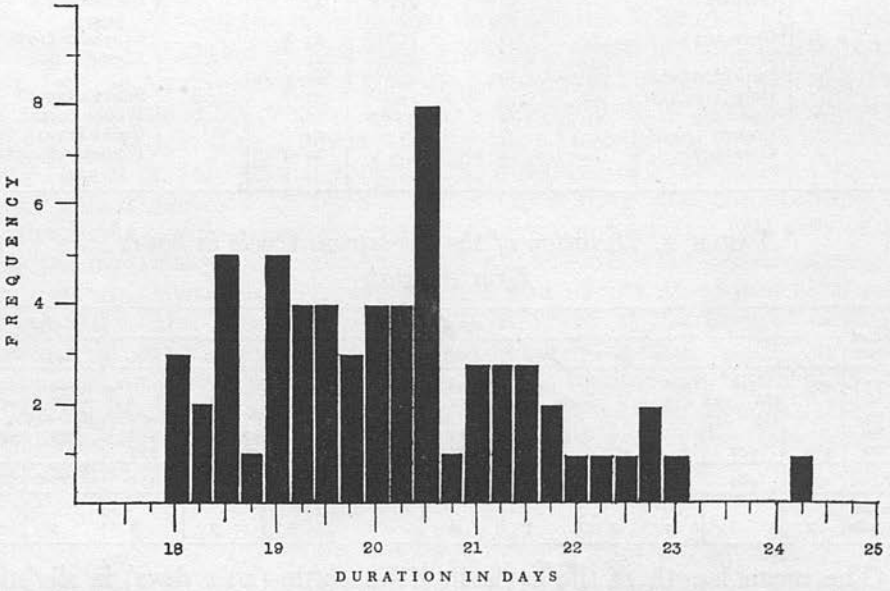

FIG. 1. Dioestrous Cycle

The Duration of Oestrus

Available records show that different workers have noted considerable variation in the duration of oestrus in the cow. It is impossible to say to what extent this is due to the methods employed in determining the existence of oestrus. The signs and intensity of oestrus vary so greatly that they are quite unreliable for this purpose. The only reliable criterion for the physiological and psychological state of oestrus is the occurrence of mating, and it is only by determining over what length of time a cow will accept the bull that the extent of oestrus can be judged.

The mean duration of 64 oestrous periods determined by Hammond in this way was 16·2 hours; the mode was 16·8 hours and the range of variation 6–30 hours. Data on the duration of oestrus in the cow is summarized in Table 4.

The most outstanding feature of the observations made by the author on the Zebu cattle was the extraordinary short duration of oestrus (Table 5). The mean length of 74 oestrous periods was 1 h. 20 min.

In 11 oestrous periods, i.e. in 14·9 per cent. of the total number of oestrous periods, single services occurred. In such cases oestrus was given the arbitrary duration of 10 min. A further check on this point is available from some of the earlier observations when two vasectomized

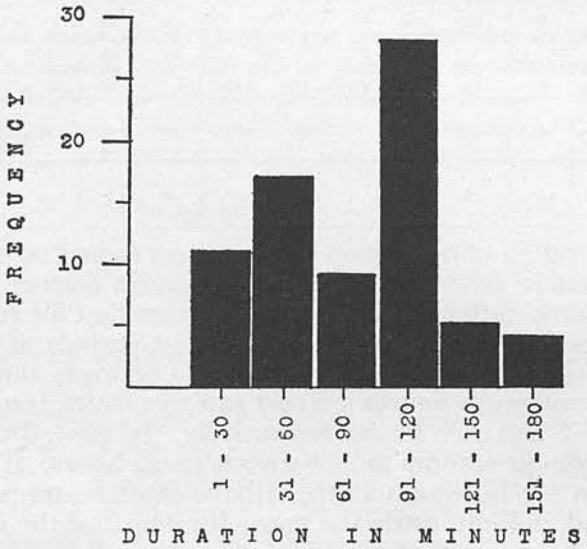

FIG. 2. Duration of Oestrus

bulls were used. At this time the procedure adopted was to remove one bull immediately after service and replace him by the other. On several occasions when this was done no second service took place, a fact which emphasizes the exceedingly brief duration of oestrus. The longest period of oestrus recorded in the 5 animals was 2 hrs. 51 min. Longer periods have, however, been recorded in two other cows, but as these animals

TABLE 4. *Duration of Oestrus*

Breed	*Country*	*No. of animals*	*No. of periods*	*Range of variation (hours)*	*Mode (hours)*	*Mean (hours)*	*Authority*
Shorthorn	Britain	15	64	6–30	16·8	16·2	Hammond (1927)
	Britain	..	12	8–21	16·6	15·7	Marshall (1924)
Zebu	Kenya	5	74	0·2–2·9	1·8	1·3	Anderson
	France	..	..	..	12–24	..	Curot (1921)
	Sicily	..	..	..	24	..	Alongi (1924)
	Italy (Umbria)	..	..	..	12–24	..	,, ,,
	Germany	..	..	..	24–48	..	Franck-Albrecht (1914)
	Germany	..	..	12–120	..	..	Schmaltz (1926)
	Germany	..	155	3–36	..	..	Weber (1911)

reacted positively to the contagious-abortion test data from them have not been included with the other results. In cow No. 12 oestrus lasted for 2 hours and 7½ hours in January and February, respectively, and in No. 41 a period of 5 hours was noted in November, and single services took place in October and January. (There is no record for December in this cow, as the vasectomized bull died of rinderpest.)

TABLE 5. *Duration of Oestrus in minutes*
Zebu Animals

No. of animal	1933										1934		
	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Average
80				10	109	118 99		165 112	60	75	99 106	106	96
72	10	120	46	111	120 135	115 106	141	77 83	81	52 45	100 110		91
90	10	10 38		10 10		10 51	41	148 75	43	95	107	109	54
31	10	10 104	45	90	113	108 171	36	47 97	57	40	152 92	109	80
66	41	10 10	72	62	150	95 97	76 115	147	55 37	158	56 108	104	82
Av. to nearest figure	18	43	54	49	125	97	82	106	55	77	103	107	81
No. of eat-periods	4	7	3	6	5	10	5	9	6	6	9	4	= 74

The shortest duration of oestrus on record seems to be that of Weber [4], who states that in cows with feeble heat-periods oestrus may last 3 hours, but the same author gives the range of variation for such cows as from 3 to 36 hours. Hammond has noted heat-periods of 6 hours' duration and Marshall a period of 8·4 hours. The mean duration of Marshall's and Hammond's figures are 15·7 and 16·2 hours, respectively, and the modes 16·6 and 16·8 hours, respectively. In general the mode has been found by most authors to lie between 12–24 hours. It is therefore the exception for European cattle to have short oestrous periods. On the other hand, in Zebu cattle the mean duration and the mode are 1·3 hours and 1·8 hours, respectively.

Hammond noted that cows have on the average a slightly longer duration of oestrus (19·3 hours) than heifers (16·1 hours); however, when the

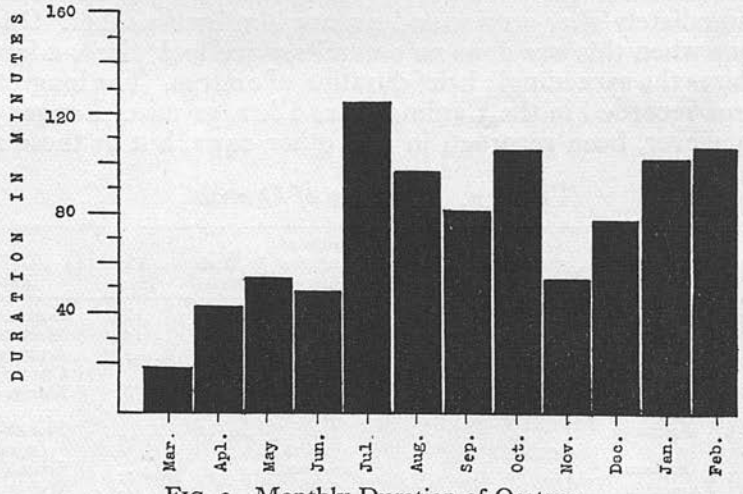

FIG. 3. Monthly Duration of Oestrus

differences in age were slight any effect was masked by individual differences. The difference in age between the Zebu heifers and cows is slight (3 and 4½ years, respectively); the duration of oestrus is similar in both heifers and cows.

The average monthly variation in the duration of oestrus is shown in Fig. 3 (see also Table 5). The average duration of oestrus is longest in

July (2·1 hours), October (1·8 hours), January (1·7 hours), and February (1·8 hours), and is shortest in March (0·3 hours).

Hammond has observed that the average length of oestrus is greatest in the summer months and shortest in the winter months, there being an average difference of 5–6 hours between the two extremes.

There is no evidence that cattle in different European countries exhibit a significant variation in the duration of oestrus. Suitable records are too few, however, to allow of the question being examined critically. Alongi [5] states that, with regard to the duration of oestrus, no significant differences exist between animals in Sicily, Italy (Umbria), and Germany.

Individual differences in the duration of oestrus in Zebu cattle are very slight, which is probably due to the shortness of oestrus in these animals. Hammond found marked individual differences, the heifers varying from 8 to 21 hours and the cows from 17 to 21 hours.

Relationship between Intensity, Duration, and Periodicity of Oestrus

It is a well-known fact that there is considerable difference in the intensity of 'heat' as shown by outward signs in different cows. Intensity is a factor which cannot be measured accurately and is thus liable to considerable error in interpretation. Nevertheless, variation in the intensity of 'heat' does occur. Weber [4] found that oestrus varied from 12 to 36 hours in cows with intense heat-periods, from 6 to 36 hours in cows with average heat-periods, and from 3 to 36 hours in cows with feeble heat-periods. Hammond has noted that during the winter months, when oestrus is short, the signs of 'heat' are slight. They are, moreover, more marked in the summer months when the duration of oestrus is greater. In sheep, Grant [6] has observed that in general long heat-periods were more intense than short ones; in particular the first heat-period of the season was usually shorter and less intense than subsequent periods. In the Zebu experimental animals the signs of 'heat' were scarcely recognizable. This fact is undoubtedly associated with the brief duration of oestrus in these animals.

The experimental data were examined for the existence of a relationship between the duration of oestrus and the duration of preceding and subsequent dioestrous cycles, but, as is shown in Table 6, no such relationship is evident. Hammond, on the other hand, has noted a correlation between the average length of the cycle and the average duration of the subsequent oestrous period, a long cycle being associated with a long oestrous period and a short cycle with a short oestrous period. Grant failed to find a correlation between the duration of oestrus and that of the preceding and subsequent cycles in the sheep; on the other hand he noted a definite negative correlation between the duration of oestrus and the duration of both preceding and subsequent interoestrous periods (i.e. the intervals between the end of one heat-period and the beginning of the next), in that the shorter the period of oestrus, the longer are the preceding and subsequent interoestrous periods. There is no correlation of this nature in Zebu cattle, which may possibly be due to the shortness of the oestrous period in these animals (Table 7).

TABLE 6. *Relation between the Duration of Oestrus and the Durations of Preceding and subsequent Dioestrous Cycles*

Oestrus (min.)	*Preceding cycles*	*Average (hours)*	*Subsequent cycles*	*Average (hours)*
10–39	8	482	13	485
40–69	13	489	13	484
70–99	14	477	12	488
100–29	19	487	15	480
130–59	7	476	7	464
160–89	1	(506)	2	502

In European cattle it is obvious, as Grant [6] has pointed out for sheep, that the interoestrous period is more variable than that of the whole cycle, since it is subject, not only to variation in the length of the cycle, but also to variation in the duration of oestrus. When oestrus is of short duration and the range of variation is not great, as in Zebu cattle, variation in its duration can have little effect on the length of the subsequent interoestrous period. In these animals variation in the length of the interoestrous period is due mainly to variation in the length of the dioestrous cycle.

TABLE 7. *Relation between the Duration of Oestrus and the Durations of Preceding and Subsequent Interoestrous Periods*

Oestrus (min.)	*Preceding interoestrus*	*Average (hours)*	*Subsequent interoestrus*	*Average (hours)*
10–39	8	481	13	485
40–69	13	488	13	482
70–99	14	476	12	486
100–29	19	486	15	479
130–59	6	480	6	465
160–89	1	(504)	2	499

The average length of the dioestrous cycle in British cattle is about 19½ days [1] and in Zebu cattle about 20 days; the mean duration of oestrus in these animals is 16·2 hours and 1·3 hours, respectively. It is therefore clear that a much greater range of variation occurs in the duration of oestrus than in the duration of the dioestrous cycle in the cow.

Summary

1. Records are given of 63 oestrous cycles in 5 animals. The duration of the cycle varied from 17·9 to 24·2 days with the mean of 20·1 days.

2. The mean duration of oestrus was found to be 1 hour 20 minutes.

3. Monthly variation in the duration of oestrus and the oestrous cycle was noted.

4. No relationship was evident between the duration of oestrus and either the duration of preceding and subsequent dioestrous cycles or the duration of preceding and subsequent interoestrous periods.

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STUDIES ON REPRODUCTION IN CATTLE

Pt. II. THE INFLUENCE OF ENVIRONMENTAL FACTORS ON REPRODUCTION

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Within recent years numerous investigations have been made on the relationship of such environmental factors as rainfall, temperature, light, and food, to reproduction, and there is now evidence to show that some of these factors do influence reproduction to some extent.

Effect of rainfall.—Baker and Ransom [1] collected field-mice (*Microtis agrestis*) from different areas over a period of two years, and in an investigation of their breeding-habits failed to find any correlation between breeding and rainfall.

Effects of temperature and light.—Under natural conditions fecundity in both sexes of the field-mouse starts in the coldest time of the year in Britain, namely February–March, and stops when the temperature is still high. Thus temperature would not seem to play an important part in influencing reproduction in this species. On the other hand, under experimental conditions, it was found that a low temperature (about 5° C.) interfered with reproduction in female mice, but the way in which it works is not known [2]. In the rat it has been shown that exposure to cold lengthens the oestrous cycle, and it is thought that this change is brought about through the lowered general metabolic level, since both body-temperature and general activity are reduced [3]. Parkes and Brambell [4] have found that mice kept at 0° C. experience regular oestrous cycles.

Rowan [5] found that artificial illumination in midwinter induced sexual activity in the Junco. Bissonnette [6] found that complete spermatogenesis and maximum testis-size could be induced in 4–6 weeks at any time from December to April in the starling by giving light exposures of 6–7 hours per night, and, conversely, that birds kept in winter light in April, May, and June, when normally in nature the testis attains full reproductive function, did not show any reproductive activity. He showed also that light and not exercise is the responsible factor, for the testis of birds on forced-exercise periods without added light actually became smaller and less active in spermatogenesis. It was, moreover, shown that the degree of stimulation depended on the intensity of the illumination and on the wave-length of the light used. Red light was highly stimulating, but green light of an equal intensity was slightly inhibitory to the activity of both testis and ovary. In the female ferret, on the other hand, Marshall and Bowden [7] found that none of the wave-lengths employed by them caused retardation of the recurrence of oestrus. The recurrence of oestrus in the ferret was greatly accelerated by light of various wave-lengths. Ultra-violet light was the most effective. Green, red, and yellow were also effective in this order.

Infra-red had a slight effect and violet a very small effect, if any at all. The effective radiation extended from red (λ6500) to the near ultra-violet (λ3650), and over this region of the spectrum intensity was more important than wave-length. There was a fairly sharp threshold on the long wave-length end of the spectrum, in that λ7500 was scarcely effective, although its intensity was high, whilst λ6500 gave a marked response.

Bissonnette [6] has found that an increase in the duration and intensity of daily exposures to light induces oestrus in the female ferret whereas reduction in the intensity and period of exposure brings about an anoestrous condition. Hill and Parkes [8] have shown that the effect of additional illumination in inducing oestrus in the ferret in winter depends on the presence of the pituitary body. These workers also found that almost total exclusion of light does not seriously affect the onset of the breeding season in this animal, which is in accordance with the fact that, histologically, breeding changes can be seen in December, long before an increase in the amount of daylight occurs [9]. Hill and Parkes therefore conclude that, whilst additional light will induce oestrus in the anoestrous ferret, the onset of the breeding season in the spring is not dependent on the increasing length of daylight. They prefer to regard anoestrus as merely one phase of the oestrous cycle that is known to depend on some inherent rhythm of the anterior pituitary body. Marshall's criticism of this is that, if there were no external factor involved in the recurrence of oestrus and the problem were merely that of the dioestrous cycle in the mouse, there is no apparent reason why the periodicity of sexual activity in the ferret should so generally conform to that of the seasons. He also points out that in comparing the dates of vulval swelling given by Hill and Parkes with those observed by Hammond and Marshall [10], there was on the average a definite lag in the time of the onset of oestrus. Bissonnette [6] also noted that there was a delay in the onset of oestrus in two hooded ferrets. It would therefore seem that there is an inherent rhythm in the sexual life of the ferret, but that light is an auxiliary factor. On the other hand, Marshall has reported the case of a blind ferret which has never experienced oestrus; and it has also been found that in blinded toads (*Xenopus*) the ovary was under-developed [11].

In observations on the breeding of the field-mouse, Baker and Ransom [1] noted a remarkable general correlation between the hours of sunshine and reproduction in this species. In general, breeding occurred only in those months in which there was more than 100 hours of sunshine. This finding was supported by an experiment in which a reduction of the daily period of exposure to light from 15 to 9 hours almost prevented reproduction in the field-mouse [12].

Nutritional Factors and the Oestrous Cycle in Animals

Under-nutrition has been associated with sterility in many animals. It has been noted that the oestrous cycle in rats is not affected by mild nutritional deficiency, but it is lengthened when the diet is very poor; the cornified-cell stage is said not to be affected [13]. In the guinea-pig,

Papanicolaou and Stockard [14], and in the rat, Long and Evans [15] have found that under-feeding increases the length of the inter-ovulation period. Macomber [16] has also noted that a reduction of 30 per cent. in the total amount of food greatly prolonged the interval between periods of oestrus in the rat. Loeb [17] found that rats underfed on a normal ration failed to ovulate, and that follicles underwent degenerative changes.

Over-feeding likewise interferes with breeding efficiency. It is often difficult to get fat animals to breed, and Hammond has shown that the duration of oestrus and the length of the cycle are shortened in animals in fat condition. A considerable amount of lipochrome has been found in the ovaries of fat heifers [18]; and Hammond suggests that the decreased length of oestrus in fat animals may be caused by a blockage of the ovarian cells with lipoids.

Evans and Bishop [19] have found that fat-free diets interfered with ovulation, but no such interference was attributed to carbohydrate-free diets.

Guilbert and Goss [20] noted that in rats the oestrous cycle was normal with a protein-intake of 7·5–8 per cent. Lowering the protein-intake to 3·0–3·5 per cent. resulted in cessation of oestrus or in long, irregular cycles. It is not known to what extent an inadequate protein-intake, permitting oestrus (but not fertility), in some animals interferes with ovarian changes, for numerous *corpora lutea* that may have been newly formed, or may have been retained from the last oestrus, were found in the ovaries of such animals 70 days after the last occurrence of oestrus. The importance of the quality of the protein has been shown by Courrier and Raynaud [21], who found that the sexual cycle did not occur on a diet deficient in lysine.

Guilbert and Hart [22] found that a low phosphorus-intake caused cessation of the oestrous cycle in rats; in some cases irregular cycles occurred and the cycles were lengthened. A change to a diet adequate in phosphorus caused oestrus to start within a few days. The importance of the calcium-phosphorus ratio, as well as the actual amount of phosphorus in the diet, is shown by the fact that oestrus ceased on a diet low in phosphorus and high in calcium, but began again on transferring the animals to a diet containing an amount of phosphorus similar to that in the previous diet but with a low calcium-content. Lack of phosphorus in the diet is said to cause cessation of oestrus in cattle [23, 24]. In South Africa phosphorus-deficiency is associated with sterility in cattle [25, 26] and in Norway, Tuff [27] has noted that a naturally occurring deficiency of calcium and phosphorus in the food causes failure to ovulate. Contrary to these findings, Boyd [28] states that experiments still in progress (no details given) on the effect of phosphorus on reproduction show that there is no interference with the rhythm of the oestrous cycle in cattle.

The inability of rats to reproduce successfully when fed on a ration of milk alone is generally thought to be due to milk being low in certain substances necessary for reproduction. Ovulation was delayed and irregular in rats on a milk diet supplemented by copper and iron, but

the addition of manganese and iodine improved the ovulatory rhythm [29]. Skinner, Van Donk, and Steenbock [30] found that even on a milk-copper-iron diet supplemented with manganese, rats showed irregular and infrequent cycles. On the addition of sucrose to this diet normal oestrous cycles (4·3 days) occurred, and on the milk-copper-iron diet with sucrose, but without manganese, the oestrous cycles were regular but longer in duration (5·4 days). The deficiency of this diet with regard to oestrus therefore seems to be one of energy, although the maximum response was not elicited without the manganese. On the other hand Orent and McCollum [31] failed to find any interference with the periodicity of oestrus on a manganese-free basal diet, and on a milk-copper-iron diet. The author has noted that injection of oestrin in the form of urine from a pregnant cow into mice in which oestrus has ceased entirely, due to an exclusive milk diet, will induce oestrus again [unpublished results]. The failure of such mice to exhibit oestrus is therefore apparently due to non-production of oestrin.

There is almost complete cessation of oestrus in rats when the sodium-fluoride intake exceeds the threshold value of 25 mg. F per kg. body-weight [32]. Rats fattened on brain and cholesterol had longer dioestrous periods, but lecithin increased the duration of oestrus [33].

Vitamin-B deficiency is associated with atrophic changes in the male and female gonads. According to Evans and Bishop [19], this deficiency results in cessation of oestrus in rats. Marrian and Parkes [34] have found that the administration of anterior pituitary substance induced oestrus in the anoestrous condition caused in the rat by inanition or vitamin-B deficiency, and it has been found that oestrin has a similar effect in vitamin-B deficiency [35, 36].

Verzar [37] has shown that the intra-peritoneal injection of vitamin E into immature female rats caused hypertrophy of the uterus, but no such effect was observed in ovariectomized animals. Male rats reared on a diet free from vitamin E had a silky infantile coat similar to that of castrated rats; the addition of vitamin E to the diet, or the injection of anterior pituitary hormone into rats on that diet, caused the development of the bristly coat characteristic of the normal male. It is therefore suggested that vitamin E is necessary for the synthesis of anterior pituitary hormone [38]. Vogt-Moller and Bay [39, 40] have used extracts of wheat-germ oil against sterility in the cow with considerable success.

In view of the relationship known to exist between the anterior pituitary and the gonads, several workers have investigated the gonad-stimulating properties of the anterior pituitary of rats on diets deficient in various factors. Marrian and Parkes [34] transplanted the pituitaries from rats lacking vitamin B into immature anoestrous rats, and obtained a response similar to that with pituitaries of normal rats. Similarly, fairly high doses of fluorine do not seem to affect the gonad-stimulating potency of the anterior pituitary in rats [32]. On the other hand, Mason and Wolfe [41] obtained a 43 per cent. greater effect with the pituitaries of rats on a diet deficient in vitamin A than with controls on normal diets.

Influence of Climatic and Nutritional Factors on the Periodicity and Duration of Oestrus in the Cow

It was thought that if environmental factors affected in any way the periodicity and duration of oestrus, a correlation between one or more of these factors and the periodicity and duration of oestrus might be evident. Accordingly, meteorological data were recorded and analyses of the pasture on which the animals grazed were made. The meteorological data are summarized in Table 1. The rainfall data are available for the

TABLE 1. *Meteorological Data*

	1933											1934	
Months	*Feb.*	*Mar.*	*Apr.*	*May*	*June*	*July*	*Aug.*	*Sept.*	*Oct.*	*Nov.*	*Dec.*	*Jan.*	*Feb.*
Rainfall (inches)	3·08	3·18	7·80	9·44	5·52	4·97	6·96	5·75	4·41	3·21	3·12	0·09	1·12
Temperature (°F.):													
maximum	..	..	..	..	..	..	75·8	78·4	78·1	79·4	83·4	90·6	90·6
minimum	..	..	..	..	..	..	56·2	56·2	58·9	68·6	58·9	57·0	61·9

whole of the experimental period, but temperature records only for the months August–February. Analyses of pasture are available for the period July–February; they are given also for the months of March and May, to show the full seasonal variation that takes place. There are no particulars for April, as the grass sample arrived in a mouldy condition and could not be analysed.

Climatic Factors

Rainfall.—It is unlikely that rainfall *per se* influences sexual function, but it might act indirectly through its effect on pasture. If this were so there would probably be a latent period before any effect would be obvious. May, with fully 9 inches of rain, was the wettest month of the year, and no increase in the duration of oestrus occurred until July, two months later, which would seem to be a long interval if rain really did exert any indirect influence. Also, there was actually a decrease in the month immediately following that of heavy rain. Moreover, the monthly rainfall decreases steadily from August to January, and the periodicity and duration of oestrus over the same period shows considerable variation. It may therefore be concluded that rainfall was not responsible for the variation noted in the periodicity and duration of oestrus.

Temperature.—In general, it can be said that climatic conditions in the tropics consist of a range from wet to dry weather, as opposed to the range from hot to cold weather in temperate climates. The stock-farm where the observations were carried out was not exposed to extreme temperatures (Table 1). Unfortunately records are available for only 7 months. They suffice, however, to show that the range of both maximum temperatures (from 75·8° in August to 90·6° in February) and minimum temperatures (56·2° in August to 61·9° in February) over the period are comparatively slight. In the wetter period of the year the temperatures would be somewhat lower. All workers are not agreed that temperature does affect reproduction, but it has been shown experimentally that only very low temperatures affect reproduction in the

mouse. The temperatures to which the experimental cattle were exposed were not extreme. Moreover, although the temperature increased steadily from August to February, both the periodicity and duration of oestrus over this period showed considerable variation. It is therefore

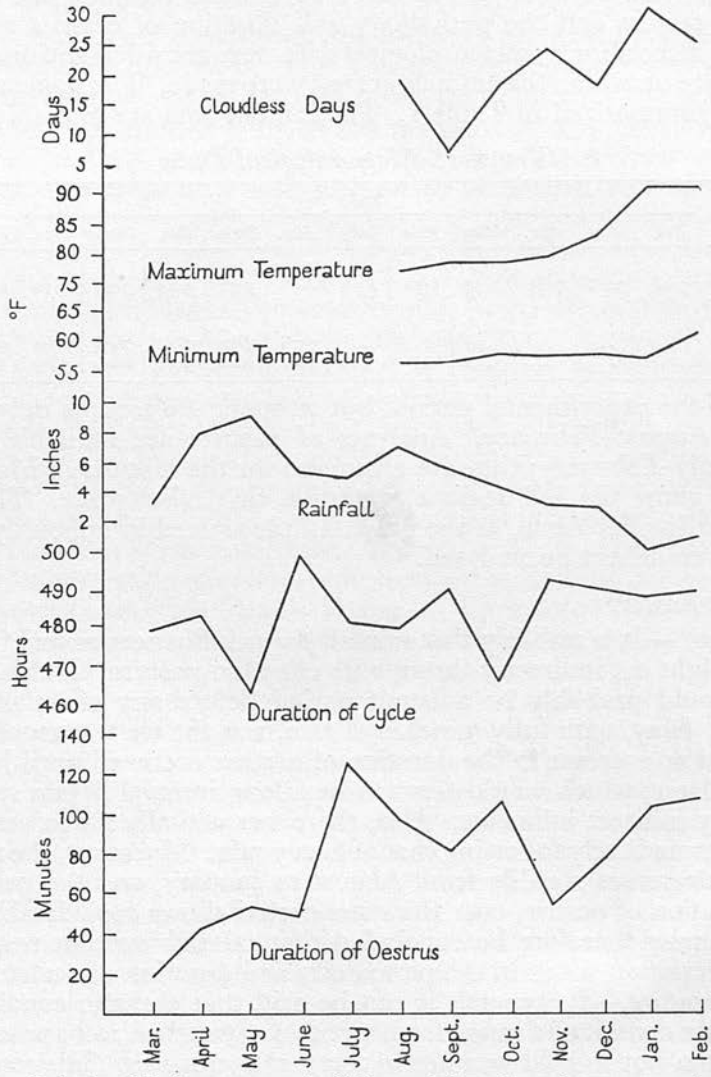

FIG. 1. Relationship between periodicity and duration of oestrus and rainfall, temperature, and cloudless days

concluded that in the present experiments temperature was not an important factor determining variation in the periodicity and duration of oestrus in cattle.

Light.—Circumstances unfortunately prevented the making of sunshine records, but a rough estimate of this factor was obtained from daily observations on the state of the weather, including the amount of cloud, and to some extent the data for rainfall and temperature (Fig. 1).

As the farm is situated almost on the equator, the length of the day varies little from season to season. Variation in the amount of sunshine must, therefore, be attributed to climatic conditions, and it may be assumed that, in general, there is less sunshine during the rainy season than in the dry season. An estimate of the state of the weather was made daily at 6 p.m., and the weather for the preceding 12 hours was also noted. For this purpose weather was regarded as: (*a*) 'cloudy', denoting a considerable amount of cloud, (*b*) 'variable sky', denoting a small amount of broken cloud, and (*c*) 'clear', when cloud was absent or at a minimum. The number of days per month with cloudy and clear weather was thus obtained (for this purpose 'variable sky' was regarded as 'clear' weather). It is admitted that this method is far from ideal, and can only give a very rough estimate of the amount of sunshine but, in the absence of more accurate data, it must for the present suffice. It will be seen from Fig. 1 that on the whole there is some similarity between the amount of sunshine, as represented by the number of days per month without cloud, and the variation in the duration of oestrus. In view of what is known of the effect of light upon reproductive functions, and bearing in mind the limitations of this method, it is apparent that the influence of light on sexual function in the cow is worthy of further investigation.

Food.—Analyses of the pasture, which formed the sole food of the animals during the experiment, is given in Table 2. It is evident that the pasture is of poor nutritive value. The contents of phosphorus, ash, chlorine, and protein, are particularly low. It is probable that the sodium-content is also low. The relationship between rainfall and pasture constituents is shown in Fig. 2.

The effect of rainfall is, on the whole, clearly reflected in the pasture constituents, an increase in the monthly rainfall being associated, in general, with an increase in the percentage of most of the constituents, and vice versa, with the exception of the month of October when, with a decrease in rainfall, an increase in the crude protein, silica-free ash, CaO and P_2O_5 occurs. The protein and silica-free ash follow each other closely, but the protein-content of the pasture increases more promptly than does the silica-free ash in response to the increased rainfall in February. Actually the P_2O_5-content parallels the protein-content much more closely than does the silica-free ash, though the variation in the P_2O_5-content is comparatively slight. Variation in the chlorine-content is likewise slight; it rises steadily till December and then falls till March, the highest value occurring in May. The CaO-content also rises till the month of October; it falls slightly in November, rises in January, falls in February, and rises again in March.

There does not appear to be any relationship between seasonal variation in the pasture constituents and variation in the periodicity and duration of oestrus. A decrease in the duration of oestrus in August–September and in October–November is accompanied by a decrease in the crude protein, P_2O_5, and silica-free ash, but while these constituents continue to fall from November–January, the duration of oestrus increases. An increase in the duration of oestrus in September–October and in January–February is associated with an increase in the crude

protein and P_2O_5 over the same period, but an increase in these constituents is accompanied by a decrease in the duration of oestrus in July–August. A decrease in the length of the cycle in November–January

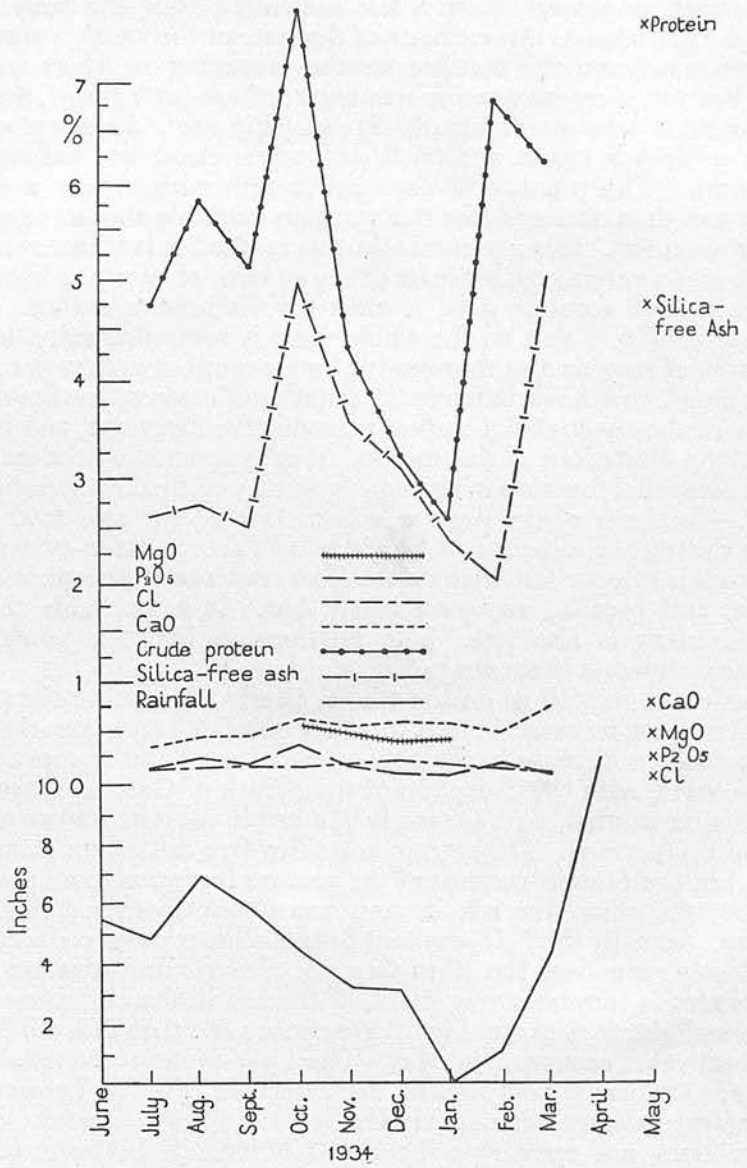

FIG. 2. Relationship between rainfall and pasture constituents

is associated with a decrease in the crude protein, silica-free ash, and P_2O_5, but an increase in the former in August to September is accompanied by a decrease in the crude protein, silica-free ash, and P_2O_5. The variation in the contents of chlorine and calcium does not correspond with variation in the periodicity or duration of oestrus. Such variation

as there is in these substances is slight, and there is no evidence that they are of great importance to reproductive functions.

Since in the method used for sampling the grass a certain amount of old grass was probably included, it is doubtful if the analyses fully represent the seasonal changes in the pasture constituents. Moreover, although no relationship is evident between the pasture constituents and the periodicity and duration of oestrus, it must be remembered that the percentages of pasture constituents shown in Table 2 do not necessarily

TABLE 2. *Analyses of Pasture Herbage*

Date of cutting	*Crude protein*	*Ether extract*	*Crude fibre*	*N-free extract*	*Silica-free ash*	*CaO*	P_2O_5	*Cl*	*MgO*
1933									
July 28 .	4·75	1·70	34·06	50·03	2·67	0·39	0·17	0·15	..
August 31 .	5·84	1·28	33·94	48·99	2·78	0·49	0·27	0·16	..
September 30	5·15	1·82	32·60	50·83	2·54	0·50	0·21	0·17	..
October 31 .	7·78	1·36	29·77	51·72	5·02	0·66	0·40	0·17	0·52
November 30	4·36	1·46	31·97	52·83	3·60	0·60	0·19	0·20	0·52
1934									
January 7 .	3·29	1·40	32·90	52·40	3·15	0·63	0·11	0·21	0·44
January 31 .	2·60	1·46	31·07	55·22	2·40	0·61	0·10	0·13	0·46
February 28 .	6·80	1·25	33·08	48·25	2·00	0·52	0·19	0·12	..
March 31 .	6·20	1·74	32·50	47·60	5·09	0·79	0·13	0·13	..
April 30 .	..	..	..	..	..	..	..	..	..
May 31 .	7·57	1·77	32·77	48·66	4·76	0·53	0·37	0·27	0·55

indicate the amounts of them consumed by the animals. No check was made on the food-consumption, and it is therefore possible that more pasture was eaten at certain times of the year than at others. It is therefore concluded that this method of correlating seasonal variation in the periodicity and duration of oestrus with seasonal variation in the pasture constituents is not one that will lead to useful results.

On the other hand, in view of what is known of the effect of shortage of protein and P_2O_5 in the diet of animals on the oestrous cycle, it is possible that the relatively small amounts of protein and phosphorus in the pasture are related to the brief duration of oestrus in the experimental animals.

Discussion

It has been shown that Zebu cattle exhibit a very short oestrous period compared with British cattle; but as the animals were living under conditions totally different from those of cattle in Britain, and on a diet poor in protein and phosphorus, the question of the relative effect of breed and nutrition on the oestrous cycle must for the present remain open.

It does, however, seem to be significant that the duration of oestrus is long at that time of the year in Kenya when there is most sunshine, namely, the dry season. The fact that the oestrous period is also long in July and October may or may not be related to the amount of sunshine. The possible significance of the relationship between the duration of oestrus and sunshine is enhanced by the facts that, (*a*) in Britain oestrus is longer in cattle during the summer months than in the winter months, and (*b*) light has a stimulating effect on reproductive function

in birds, mice, and ferrets. If light as a stimulus to reproductive function is to be regarded as a factor of universal importance, it is generally considered probable that it is the change in the light ration and not the actual amount which is the decisive factor, for most breeds of sheep, for example, commence their mating season when the hours of daylight are decreasing in the autumn, and mating stops with increasing daylight in the early spring.

Variation in the duration of oestrus can probably be related directly to variation in the production of oestrone, the hormone responsible for the oestrous phenomena in animals, and this in turn to variation in the gonadotropic activity of the anterior pituitary. It has been shown that hypophysectomy in the ferret prevents the response to light treatment which is obtained in the normal animal [8]. It is therefore possible that light is capable of stimulating the anterior pituitary in cattle, possibly through the eye.

Summary

1. Present knowledge on possible relationships between rainfall, temperature, light, food, and reproduction is reviewed.

2. The possible influence of climatic and nutritional factors on the periodicity and duration of oestrus in the cow was investigated. No correlation was found for rainfall or temperature, but some indications were found of a possible correlation between sunshine and variation in the duration of oestrus.

3. Analyses of the pasture (which formed the sole food of the experimental animals), cut at monthly intervals, were made for the period July 1933 to May 1934. No relationship was found between seasonal changes in pasture constituents and the periodicity and duration of oestrus. This line of investigation is unlikely to lead to useful results.

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ARTIFICIAL INSEMINATION OF SHEEP

I. PRELIMINARY INVESTIGATION ON ITS APPLICATION TO SHEEP BREEDING IN KENYA

BY

JAMES ANDERSON, M.R.C.V.S.

FROM THE JOURNAL OF AGRICULTURAL SCIENCE
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CONTENTS OF VOL. XXVII, PART 1. JANUARY 1937

PAGE

1. WATSON, S. J. and FERGUSON, W. S. The chemical composition of grass silage. (With three text-figures) 1
2. DILLON WESTON, W. A. R., HANLEY, F. and BOOER, J. R. Seed disinfection. II. Large-scale field trials on the disinfection of seed corn with mercury dust disinfectants 43
3. BRETT, C. C., DILLON WESTON, W. A. R. and BOOER, J. R. Soil disinfection. III. Experiments on the germination of peas. Seed protection by the use of disinfectant dusts containing mercury. (With Plate I) . 53
4. WATSON, S. J. and FERGUSON, W. S. The losses of dry matter and digestible nutrients in low-temperature silage, with and without added molasses or mineral acids 67
5. MANN, HAROLD H. The character of barley grown on soil made acid with sulphate of ammonia. (With Plate II) 108
6. BEATER, B. E. Improved technique in grading of coarse and fine sands during mechanical analysis of soils. (With one text-figure) . . . 123
7. HALNAN, E. T. Digestibility trials with poultry. VII. The digestibility of wheat offals, with a note on the apparent discrepancy between the digestibility coefficients and nutritive values of these products . . 126
8. HALNAN, E. T. Digestibility trials with poultry. VIII. The digestibility of dried molassed sugar-beet pulp 137
9. ANDERSON, JAMES. Artificial insemination of sheep. I. Preliminary investigation and its application to sheep in Kenya 143

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ARTIFICIAL INSEMINATION OF SHEEP

I. PRELIMINARY INVESTIGATION ON ITS APPLICATION TO SHEEP BREEDING IN KENYA

By JAMES ANDERSON, M.R.C.V.S.

Division of Veterinary Research, Experimental Station, Naivasha, Kenya Colony

In recent years artificial insemination has been very successfully applied to livestock breeding in Russia. In 1932, 920,000 ewes were inseminated with a conception rate of 82·4 per cent, which compares very favourably with the percentage of conceptions (79·6) in 1,560,000 ewes served normally (Ozin & Parsutin, 1934). In 1933 the percentage of conceptions in 1,500,000 sheep inseminated was equal to, or above, that of normal service (Parsutin, 1934). The percentage of fertilization after artificial insemination in 31,911 Karakul ewes was 79·42 (Kuznetzova, 1932). On the other hand Demidenko *et al.* (1933) with a flock of 3459 ewes obtained 57·8 per cent of conceptions following artificial insemination as opposed to 79·0 after normal service. It is probable that the successful application of artificial insemination to livestock breeding in the Colonies and other overseas countries would be of considerable value, and the experiment which is described in this paper was designed to explore these possibilities as far as sheep breeding in Kenya is concerned.

Experimental material and methods

The technique adopted was essentially that of Russian workers described in the monograph of the Imperial Bureau of Animal Genetics (1933). The collection of sperm was easily effected by means of an artificial vagina (Walton's pattern). Prior to use, the inner surface of the rubber lining was swabbed with 65 per cent alcohol, allowed to dry and smeared with white vaseline. The space between the cylinder and the inner tube was filled with warm water at a temperature of about 40–50° C. The correct distension of the tube was then obtained by blowing air into the space between the cylinder and the tube. The artificial vagina on account of its small size cools down fairly quickly and it is therefore considered advisable to warm the cylinder with hot water before finally filling it with water at a temperature of 40–50° C.

In the large majority of cases the ram from which it was desired to

collect sperm was placed with a ewe on heat, which had been picked out by a "teaser" ram. It does not, however, seem to be essential that a ewe used for this purpose should be on heat, since on several occasions a ewe which was not on heat has been used successfully. If the ewe is held to prevent her moving, a ram accustomed to the procedure will mount her.

Sperm was microscopically examined immediately after collection for motility and density. It was then diluted and examined microscopically as a check on the effect of the dilutor on the sperm; a further microscopic examination was made on any sperm remaining after the insemination had been carried out. No specimens of sperm from the ram chosen for the insemination had to be discarded because of poor quality. The maximum time that elapsed between collection of sperm and the end of the insemination was an hour, but in the majority of cases the insemination was usually completed in about half an hour.

The dilutor used was GPS-2 (Milovanov & Selivanova, 1932), which has the following composition:

Solution I (g: per litre of water)	
Anhydrous glucose	64·0
KH_2PO_4	1·7
Solution II (g. per litre of water)	
$Na_2HPO_4 12H_2O$	41·6
$CaHPO_4$	0·1
$MgHPO_4$	0·1

These solutions were made up with distilled water that had been boiled to expel CO_2. The equivalent amount of anhydrous disodium hydrogen phosphate (B.D.H.) was used in place of the $Na_2HPO_4 12H_2O$. The *p*H of the dilutor was tested with a phenol red capillator. This should be 7·6. The dilutor was kept in test-tubes or flasks plugged with cotton wool. Sterilization was carried out immediately after preparation in a steam sterilizer; this was done for 20 min. on three successive days. For use, equal amounts of solutions I and II were taken and mixed, sufficient dilutor being prepared according to the dilution required. Flasks were sterilized daily after withdrawal of the required amount of dilutor. For the first insemination 151 ewes were inseminated with sperm diluted $\times 10$ and the remaining fifty-one with sperm diluted $\times 2$ and $\times 4$; for succeeding inseminations dilutions of $\times 2$ and $\times 4$ were used.

The instruments used for insemination were disinfected in the following manner: the speculum was swabbed with 65 per cent alcohol

and dried thoroughly with sterile cotton-wool swabs. After each ewe the speculum was washed in water, if necessary, and then swabbed. A 2-ml. glass syringe and vulcanite nozzle was used for the introduction of the sperm. The syringe was sterilized in a hot-air oven, and the vulcanite nozzle was sterilized by filling it with 65 per cent alcohol which was allowed to remain in it for a few minutes. It was then thoroughly washed out with dilutor. After inseminating a ewe, the outside of the nozzle was disinfected by swabbing with 65 per cent alcohol and dried with sterile cotton-wool swabs.

For the actual insemination the ewe was stood on a table or placed over a broad wooden rail about 3 ft. 6 in. high. The vulvar region was washed with a sponge and water. The speculum was disinfected and inserted into the vagina and the cervix exposed. The cervix was illuminated with a battery lamp fixed to the forehead of the operator. The tip of the vulcanite nozzle was now inserted into the cervix and 0·2 ml. sperm introduced. If the nozzle is introduced too far the pressure of the cervical tissue against the tip of the nozzle will prevent the exit of the sperm, but this can be avoided by withdrawing the nozzle slightly. The type of speculum used is of some importance and it is recommended that the Russian model ewe speculum be used (Holborn Surgical Instrument Co.).

The animals used in this experiment were a flock of 202 "high-grade" Merino ewes, and the ram was a pure-bred Merino. This ram was used for all the ewes.

Insemination was carried out once daily from 18 December 1935 to 29 February 1936.

Four vasectomized and vasoligated rams were used to pick out the ewes on heat. These rams were put with the ewes at 6 a.m. every morning and insemination was usually carried out about 9 a.m. The ewes were under supervision during this period and, in addition, the rams were "keeled" with raddle. Immediately a ewe was served, she was removed from the flock to prevent the ram serving her repeatedly. When a considerable number of the ewes had been inseminated, the number of "teaser" rams was reduced to three and later to two.

RESULTS

No. of inseminations ...	1	2	3	4
No. of ewes	202	91	34	17
No. of ewes "settled"	111	57	(17)	—
% of ewes "settled"	54·9	62·6	—	—

The number of ewes that "settled" (as indicated by those that did not return to the ram) was 168, i.e. 83·2 per cent for the first two inseminations. Of the thirty-four ewes inseminated for the third time seventeen had returned to the ram by the end of February when the vasectomized rams were taken out. The number of ewes that lambed was 141, and nine ewes which died were found to be pregnant on post-mortem examination. Thus 74·3 per cent of the ewes conceived. The total number of live lambs, dead lambs and foetuses was 188 (93·1 per cent).

Discussion

Before the beginning of an insemination season a ram or rams of a suitable type should be selected. These rams should be thoroughly tested for sexual vigour and sperm production. Rams vary considerably in sexual vigour and in the ease and rapidity with which they serve a ewe. Sexual vigour can be judged by observing the behaviour of a ram when with an oestrous ewe, e.g. when attempting to collect sperm with the artificial vagina. A ram when presented with a ewe after a long period of rest may be over-excited, and may not experience a proper or complete ejaculation. Several examinations should therefore be made on such rams. The volume of the sperm produced, the physical characters, motility and density must be determined. The average volume of sperm produced by the ram is said to be about 1·6 ml., but in the present experiment the average volume was approximately 0·5 ml. The quality of the sperm can be readily determined by naked-eye examination. A good specimen of sperm is of a creamy colour and of thick consistence. Motility can be noted in a good specimen without recourse to a microscope, but it is advisable in all cases to make a microscopical examination as well. If this is done immediately after collection of the sperm the motility can be judged on an ordinary slide examined, but it is best to use a hanging drop preparation since sperm dries up rapidly with loss of motility at room temperature (18–20° C.) Sperm diluted for use should also be examined before and, if possible, after use. This is of particular importance when a new dilutor is being used. In one instance a dilutor used by the author seemed normal when the diluted sperm was examined before the insemination was carried out, but it had killed all the sperm after the lapse of half an hour. This examination is recommended as a routine procedure, but with a dilutor that has been thoroughly tested it should not be necessary. The density of the sperm can, as has been mentioned, be determined from its physical appearance, and this is confirmed by the microscopical examination. The technique

for a sperm count has not been fully investigated by the author but, using a haemocytometer and a dilution of $\times 200$, the average for twenty-four specimens from six rams was 2,907,775 per c.mm.

Several lots of dilutor were made with water distilled in a copper still. These dilutors had varying effects on the sperm; some were toxic to varying extents and others again were normal. Water for the preparation of dilutors is now distilled in glass vessels and this toxic effect has been obviated. One lot of dilutor, which was filled into test-tubes from an automatic pipette with rubber junctions, also proved toxic. This toxic effect does not arise if a glass burette is used.

Since in many cases on account of the unsuitable type of speculum used sperm could not be injected directly into the cervix, the results obtained in this experiment cannot be regarded as an indication of what might be expected using a suitable speculum and introducing the sperm directly into the cervix. A suitable speculum was used for the third and fourth inseminations but the percentage conception after the third insemination is at the most 50 per cent. However, this group of thirty-four ewes doubtless contained a number that had failed to conceive previously on account of sterility.

It is known that the introduction of sperm into the vagina gives only half the percentage of conceptions as the same quantity introduced into the cervix. The injection of 1 ml. of sperm into the vagina is considered to be the equivalent of 0·2 ml. into the cervix. In the present experiment the injection of sperm into the vagina, instead of into the cervix in a number of cases, is considered to be, at least to some extent, the reason for the low percentage of conceptions following the first insemination in the present experiment. Where possible in these cases larger quantities of sperm were injected into the vagina but it was seldom that sufficient sperm was available to permit of the injection of 1 ml.

The dilutor GPS-2 in a dilution of $\times 8$, used in mass work, has given a percentage of fertilization of from 65–80 (Milovanov & Selivanova, 1932). In actual practice a dilution of $\times 8$ seems to be the optimum for this dilutor. Kuznetzova (cited by Milovanov & Selivanova, 1932) gives the following data on the percentage of "settling" in 263 ewes with various dilutions of GPS-2.

Degree of dilution	0	2	4	8	16	32	64
% of "settling"	79·2	80·0	80·0	83·7	59·2	62·0	59·0

The Merino is not highly fertile compared with other breeds of sheep. In Australia it is said that the lambing percentage in the vast

majority of flocks is well under 100, and it rarely exceeds 90. During unfavourable seasons it may fall to under 50 and even less (Gilruth, 1936). These figures are estimates from counts made at marking. The actual lambing percentage would certainly be greater. In the South African Merino fertility is also comparatively low, probably not exceeding 90 per cent (Quinlan *et al.* 1932). The Kenya Merino is a "high-grade" sheep that has been developed by crossing local Masai ewes with imported pure-bred Merino rams. Nothing is known of the fertility of Masai sheep and there are no accurate records of fertility in the high-grade Merino flocks. In the larger Merino flocks in Kenya, numbering perhaps 5000–10,000 breeding ewes, 80 per cent is considered a good lamb crop. This again is merely an estimate made at "tailing". The lambing percentage in the experimental group of 202 ewes that were artificially inseminated therefore compares quite favourably with that after natural mating.

The ewes in this experiment were inseminated once daily, and probably therefore comprised ewes at all stages of heat. It is necessary to know if insemination at the beginning of heat is as effective as insemination at the end of heat. This question is related to that of the vitality of spermatozoa in the female genital tract, and with the time of ovulation in relation to the duration of oestrus. The Russian view is that one insemination from the beginning of heat to 30 hours is adequate (Milovanov, 1934). The work of Quinlan *et al.* (1932, 1933), who investigated this problem from a different viewpoint, namely vitality of spermatozoa in the female genital tract, is in agreement with this Russian view. These workers state that the cervical canal appears to be the natural habitat of the sperm from which small numbers are continually passing forward to the uterus and Fallopian tubes. Some living sperms may be found in the cervical canal up to 48 hours after service, and 24 hours after service sperms are still numerous and actively motile.

The experimental determination of the optimum time of artificial insemination has been investigated by different workers. The percentage conception using dilutor GPS-2, with insemination carried out at the following intervals from the beginning of heat, namely, 2–18, 18–26, 26–42, 30–46, 42–50 and 46–54 hours, was 80·99, 84·90, 48, 38·65, 14·5 and 2·6 respectively (Kardymocic *et al.* 1934). Zajac (1935) gives the following results: the percentage conception at 8, 16, 24, 32, 40, 48 and 56 hours after the beginning of heat were 70·2, 82·5, 85·8, 82·9, 76·9, 66·7 respectively. The optimum time for insemination in the first experiment was 18–26 hours after the beginning of heat, and in the second 24 hours, though by the thirty-second hour the percentage of conceptions (82·9)

was still high. Observations on the duration of oestrus in high-grade Merino sheep in Kenya so far indicate that the majority of heat periods are of less than 30 hours' duration. It would therefore appear that a single insemination during a heat period would be effective in these sheep, but this question will require further investigation.

PRACTICAL APPLICATIONS

The practical applications of artificial insemination to livestock breeding are too well known to require more than a brief mention here. In particular, however, it is desired to indicate briefly the application and value of artificial insemination of sheep in Kenya and other colonies.

The application of artificial insemination to sheep breeding in Kenya would benefit both the European farmer and the native. In Kenya 3 per cent is the usual percentage of rams in European-owned flocks. In the larger flocks it is impossible to provide anything approaching this number of pure-bred rams, and consequently a large number of grade rams have to be used. If artificial insemination were used, grade rams could be entirely dispensed with and the number of pure-bred rams required could be considerably reduced. Moreover, a much better type of ram could be used than is at present possible. On the Experimental Station, Naivasha, eighteen rams were kept for a flock of about 600 ewes. Of these rams eleven were pure-bred and seven "grades". In future it is intended to keep one pure-bred ram of a very much better type than is at present available for this flock of ewes, which with the addition of 300 native sheep will number approximately 900 ewes. This number of ewes does not by any means represent the maximum number that can be inseminated with one ram. A Rambouillet ram has sired in one season a crop of 2580 lambs (Parsutin, 1934). Two other specially selected rams have sired 2733 and 1403 ewes respectively during a mating season with over 70 per cent of fertilization (Kuznetzova, 1932).

Native sheep are of small value, averaging probably about 5*s*. per head in Kenya. They supply at present a purely local demand for slaughter. In the event of an export trade for mutton being developed there is the possibility of crossing these sheep with rams of mutton breeds, but this would have to be carried out in relation to the available plane of nutrition. There would seem to be a greater possibility of developing a cross-bred native wool-bearing sheep. It is known that "grading" Masai ewes with Merino rams results in a good wool type of sheep. The improvement of sheep in the Masai Reserve would now seem to be a practical proposition. In the 1931 *Report* of the Kenya Depart-

ment of Agriculture it is estimated that there are a few million native-owned sheep in the Northern Frontier Province. This is a very dry area, and would probably be well suited to a cross-bred Merino sheep. A flock of 200 Masai ewes, and 100 Black-headed Persian ewes which are found in the Northern Frontier Province have been purchased for this station, and it is proposed to cross these sheep with a pure-bred Merino ram in order to determine the wool characteristics of succeeding generations. In time, therefore, the potential value of such cross-bred sheep will be fairly well known.

SUMMARY

An account is given of a preliminary experiment on artificial insemination of cross-bred Merino sheep in Kenya. 202 ewes were inseminated and 74·3 per cent conceived. The practical application of artificial insemination to sheep breeding in Kenya is discussed.

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BY

JAMES ANDERSON, B.Sc., Ph.D., M.R.C.V.S.

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CONTENTS OF VOL. XXVIII, PART 1. JANUARY 1938

PAGE

1. SMITH, H. FAIRFIELD. An empirical law describing heterogeneity in the yields of agricultural crops. (With seven text-figures) 1
2. MARSHALL, W. and CRUICKSHANK, D. B. The function of the cuticle in relation to the porosity of eggs. (With Plate I and three text-figures) . 24
3. WOODMAN, H. E. and EVANS, R. E. The mechanism of cellulose digestion in the ruminant organism. IV. Further observations from *in vitro* studies of the behaviour of rumen bacteria and their bearing on the problem of the nutritive value of cellulose 43
4. ANDERSON, JAMES. Ovulation in the ewe 64
5. RICHARDSON, H. L. The nitrogen cycle in grassland soils: with especial reference to the Rothamsted Park grass experiment. (With nine text-figures) 73
6. MACIRONE, C. and WALTON, ARTHUR. Fecundity of male rabbits as determined by "dummy matings". (With Plate II and four text-figures) . 122
7. FOREMAN, F. W. Observations on the proteins of pasturage. Phosphorus and protoplasm 135

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OVULATION IN THE EWE

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Numerous investigations on the occurrence of ovulation in the ewe have been made by different workers. Apart from its theoretical interest the accurate determination of the time of ovulation is of practical importance in ascertaining the best time for mating. The observations reported in this paper were undertaken to assist in determining the optimum time for the introduction of sperm in artificial insemination experiments.

The earliest record of ovulation in the ewe appears to be that of Bischoff (1844), who observed that ovulation was spontaneous and occurred 24 hr. after the onset of oestrus. Marshall (1903) also concluded that ovulation was spontaneous. Ivanow (1913), cited by Grant (1934), showed that coitus had no effect on the time of ovulation. Ivanow also made numerous observations on the time of ovulation in relation to the period of oestrus. He found in a Russian breed that one ewe out of forty-three killed during the first 24 hr. of oestrus had ovulated, forty-two out of forty-six killed during the second 24 hr. and eight out of nine killed during the third 24 hr. had ovulated. Twenty-six out of twenty-eight ewes killed 1–24 hr. after the end of heat had ovulated; thirty ewes killed on the second day, twenty-eight on the third day and twenty-eight on the fourth day after the end of heat had all ovulated. Ovulation thus occurred during the 24–48 hr. period of heat in the majority of cases, and rarely in the first 24 hr. of heat; also the majority of ewes had ovulated within 24 hr. after the end of heat.

Quinlan & Maré (1931) stated that ovulation rarely takes place before the 36–40th hour of oestrus in the Merino. In one ewe killed at the 40th hour of oestrus the follicle had ruptured a very short time before slaughter, and in another ewe killed at the 41st hour of oestrus the follicle had just ruptured. All ewes killed 12 hr. and more after the end of oestrus had ovulated. The authors concluded that there appears to be little doubt that ovulation occurs towards the end of oestrus. They

also stated that the duration of oestrus appears to depend on the time of rupture of the follicle, and that delay in rupture appears to explain the prolonged oestrus observed occasionally.

Cole & Miller (1932) found that ovulation had not occurred in six Rambouillet ewes killed during the first day of oestrus, but eight out of twelve killed on the second day had ovulated. In a later paper these authors observed that ovulation occurs about the 25th hour of oestrus (1935). Five ewes had not ovulated by the 22nd hour, and one ewe had not ovulated by the 30th–32nd hour. They stated that it is agreed by all the more recent investigators that ovulation occurs spontaneously during the second half of the oestrous period, and that judging from their own results it occurs rather uniformly between the 22nd and the 30th hours of oestrus.

Allen *et al.* (1931) did not observe ovulation before the 24th hour after the first appearance of oestrus; unsegmented ova were found in the oviduct between the 24th and 50th hours.

McKenzie *et al.* (1933) found that ovulation had not occurred in seven ewes at 15·5, 19·3, 22, 23, 24·1, 28 and 49·5 hr. respectively after the onset of oestrus, and had occurred in twenty-four ewes observed from 23·7 to 49·5 hr. after oestrus began. Ovulation was actually observed in three instances at 29·8, 34·5 and 37·1 hr.

Clark (1934) found that ovulation occurred rather late in oestrus. Stieve (1934) examined eight ewes immediately before, during and after the end of heat. Ovulation had occurred in three ewes examined after the end of heat. It was concluded that heat persists as long as the ovary contains a mature follicle and ceases when the follicle has ruptured. Grant (1934) killed five ewes during oestrus, at 1, 6, 8, 18 and 24 hr. after the onset of oestrus, and only the ewe killed at 18 hr. had ovulated. It would appear that rupture may occur earlier (at 18–24 hr. after the beginning of heat) in Scottish sheep than in other breeds.

Green & Winters (1935) from an examination of thirteen grade Shropshire ewes found that ovulation occurred late in the heat period as the animal was passing from heat. Ovulation had occurred in five ewes that were off heat when killed and had not occurred in two ewes killed at 8 and 12 hr. after the beginning of heat. Evans & Miller (1935) found a fertilized ovum in the Fallopian tube 24 hr. after oestrus was first noted.

The variation in the time of ovulation noted by different authors may be due to breed and possibly environmental influences, as well as to the accuracy with which the stages of oestrus were timed. It seems,

however, that in the majority of cases ovulation occurs towards the end of heat or shortly afterwards, and that it rarely occurs before 24 hr. after the onset of heat.

Material and methods

The experimental animals were high-grade Merino ewes and an indigenous breed known as Masai sheep. Vasectomized rams were run with the flock, which was under constant supervision, to pick out ewes on "heat". The time of onset of oestrus was thus known accurately. When a ewe came on heat she was placed in a separate paddock and tested by the "teaser" ram every three hours. Later, tests for heat were made at half-hourly or shorter intervals. It was thus possible to determine with a fair degree of accuracy the stage of oestrus or met-oestrus at which the ovaries were examined. When the ovaries of a ewe that was on heat were to be examined the examination was conducted as quickly as possible after the last service to ensure that the ewe would still be on heat at the time of examination.

All ovarian operations were made by laporotomy, in the majority of cases under nembutal anaesthesia. Some ewes were therefore used several times. Prior to rupture, elevation of a small papilla on the surface of the follicle occurs as noted by Grant (1934). When rupture was very recent oozing of blood from the point of dehiscence was invariably noted. When ewes were operated on during oestrus a further examination was made after 2–3 days to discover whether or not ovulation had occurred.

Results

The results are presented under three headings: examination of ovaries of (1) ewes during oestrus, (2) ewes after the end of oestrus, and (3) ewes with short oestrous periods.

Examination of ovaries of ewes during oestrus. Eight laporotomies were performed on six ewes in this series, at intervals ranging from 19½ to 50 hr. after the beginning of oestrus. All the ewes stood well for the ram at the last service, and since the interval between this service and the time of examination of the ovaries was short in most cases it can be concluded that the ewes were actually on heat at the time of examination.

In none of these ewes had ovulation occurred at the time of examination, but all had ovulated when examined 2–3 days later. In five cases the examination was conducted at intervals of from 27½ to 50 hr. after the beginning of oestrus. In contrast, all the ewes examined during this

interval which had been off heat for more than 1 hr. had ovulated (Table II). There was considerable variation in the size of the mature follicle in these ewes which was probably due to the different stages of oestrus at which they were examined. No papilla was observed in any of the follicles. It was therefore concluded that ovulation in the ewe does not occur during oestrus.

Table I. *Examination of ovaries of ewes during oestrus*

Date	Ewe	No. of hours since onset of heat	State of follicles	Interval between last service and examination of ovaries
6. iv. 36	34	19½	No large follicle	2½ hr.
27. iv. 36	33	22	,,	15 min.
20. v. 36	34	24¼	,,	2 hr.
19. v. 37	M 22	27½	1 large follicle	25 min.
30. v. 37	220	30	No large follicle	13 min.
29. v. 37	M 23	37½	1 large follicle	37 min.
9. xii. 36	220	44	No large follicle	15 min.
20. vi. 37	M 8	50¼	,,	10 min.

Examination of ovaries after the end of oestrus. Fifteen laporotomies were performed on twelve ewes at intervals ranging from 30 hr. 45 min. to 73 hr. after the beginning of oestrus. In most cases the operation was performed as soon as possible after the end of oestrus, which was known to within half an hour. The time relationship of ovulation to the beginning and end of oestrus is thus available.

Table II. *Examination of ovaries of ewes after the end of oestrus*

Date	Ewe	Duration of oestrus hr.	Interval between examination of ovaries and: Onset of heat hr.	Onset of heat min.	End of heat hr.	End of heat min.	State of follicles
24. vi. 37	71	30	30	45	0	47	1 papilla ruptured during examination
16. xi. 36	394	30¼	31	25	1	14	1 follicle ruptured
29. iv. 36	34	26⅓	34	0	8	0	2 follicles ruptured
18. xi. 36	46	33½	34	0	0	33	Small papilla—not ruptured
3. vi. 37	M 12	33½	34	17	0	41	1 follicle ruptured
30. xii. 36	M 24	34¼	35	20	1	10	,,
16. xi. 36	235	35	36	0	1	31	,,
16. xi. 36	379	37¾	38	55	1	10	,,
11. v. 36	34	38	42	0	4	0	,,
29. xii. 36	M 22	41¼	42	17	1	8	,,
11. v. 37	M 23	44¾	45	45	1	5	,,
16. i. 37	M 24	45½	46	35	1	20	,,
23. xi. 36	436	34¼	47	0	13	0	,,
25. vii. 36	436	—	72	0	—		,,
24. vii. 36	511	—	73	0	—		,,

In all but two of these ewes the follicle had ruptured. Ovulation occurred as early as 41 min. after the end of heat and in every case had taken place 65 min. or more after the end of oestrus. In the ewes examined shortly after the end of oestrus ovulation had occurred recently.

There is considerable variation in the duration of oestrus in these ewes, and no correlation is evident between the time of ovulation and the length of the interval between onset of oestrus and examination of the ovaries. It appears that ovulation is related to the end rather than to the beginning of oestrus and that it occurs shortly after the cessation of oestrus. Ewe No. 46, for example, had not ovulated when examined 33 min. after the end of heat. There was, however, a small papilla on the follicle, and it is probable that rupture was imminent. During the examination of ewe No. 71, 47 min. after the end of heat, the small papilla on the capsule ruptured. This may, however, have been due to manipulation of the ovary. On the other hand, in ewe No. M 12 the follicle had ruptured when examined 41 min. after the end of heat.

Examination of ovaries of ewes with short oestrous periods. The maximum duration of oestrus in this series is 21 hr., which is considerably shorter than the average of the Merino in Kenya (unpublished results). In these ewes it was found that there was no close correlation

Table III. *Examination of ovaries of ewes with short oestrous periods*

Date	Ewe	Duration of oestrus hr.	Interval between examination of ovaries and: Onset of heat hr.	Onset of heat min.	End of heat hr.	End of heat min.	State of follicles
5. vi. 37	352	11¼	19	21	8	0	1 large follicle
3. vi. 37	370	21	22	25	1	27	,,
19. xi. 36	510	19	23	0	4	0	Small papilla not ruptured
14. xi. 36	39	19¼	23	0	4	30	1 large follicle no papilla
6. vi. 37	353	18	25	37	7	37	1 follicle ruptured very recently
21. v. 36	35	8¼	27	30	19	30	1 follicle ruptured
2. v. 36	35	14	29	0	15	0	,,
13. iv. 36	35	20	29	30	9	30	,,

between the end of oestrus and the time of ovulation as has been noted in ewes with longer oestrous periods. The earliest time at which ovulation was noted was 25 hr. 37 min. after the beginning of oestrus. In this ewe the ovaries were examined 7 hr. 37 min. after the end of heat and ovulation had just occurred.

In three other cases in which the ovaries were examined 27½, 29 and 29½ hr. after the onset of oestrus and 19½, 15 and 9½ hr. respectively after the end of heat, ovulation had occurred. In ewe No. 510, examined 23 hr. after the onset of oestrus and 4 hr. after the end of oestrus, there was a small papilla on the follicle and rupture was probably imminent, but in ewe No. 39, examined at much the same time, there was no sign of imminent rupture. It would thus appear that a minimum period of approximately 23–25 hr. must elapse after the onset of oestrus before ovulation occurs.

DISCUSSION

There is little change in the size of the follicle during interoestrum. Grant states that apparently during interoestrum one or more follicles grow to a certain size, and at the beginning of oestrus the largest follicles start to grow rapidly. Casida & McKenzie (1932) found that there was a gradual increase in the mean diameter of the follicle during the first third of the cycle, but the follicle was just as large at approximately the mid-point of the cycle as just before ovulation. Grant did not confirm Quinlan & Maré's statement that rapid enlargement of the follicle destined to rupture at the next ovulation occurs shortly after ovulation. The author noted that in the early part of oestrus it was often impossible by naked-eye examination to determine which follicle was going to rupture. Later in oestrus there was usually one follicle which was obviously larger than the others. The appearance of the maturing follicle prior to rupture observed by the author was very similar to that observed by other workers. The capsule of the follicle becomes thinner as ovulation approaches and the follicle acquires a darker appearance. The vascularity of the capsule appears to increase and capillaries in the capsule can usually be seen. Before rupture occurs a small papilla is elevated on the surface of the capsule. The first reference to this papilla appears to have been made by Grant. It was noted by the author in every case in which rupture was about to occur.

According to Quinlan & Maré the point of rupture is indicated by an opening with somewhat rugged borders. The border is stained with coagulated blood and a small blood clot rapidly closes the opening. Cole & Miller (1935) stated that the occurrence of ovulation is readily determined by prominent rupture points protruding from the surface of the ovary. A blood clot appears at the point of rupture. Green & Winters (1935) stated that at the point of rupture a red area denoting the location of the former follicle is observed with little difficulty. In

the author's experience the first sign of rupture is oozing of blood from the papilla on the surface of the follicle. It would appear that ovulation in the ewe is a quiet gradual process, similar to that observed by Walton & Hammond (1928) in the rabbit, and not a sudden ejaculation of follicular contents. This view agrees with Hartman's statement that ovulation is not a cataclysmic process but a gradual opening of the follicle (1932). On the other hand, Hill *et al.* (1935), from a cinematographic study of ovulation in the rabbit, concluded that ovulation is truly explosive in nature. It is conceivable that the oozing of blood from the follicle of the ewe might appear to be more in the nature of an ejaculation if examined under greater magnification such as used by these authors in the rabbit.

Ovulation did not occur in any of the ewes examined by the author while they were still on heat. There are some records of ovulation occurring during heat (Quinlan & Maré, 1931; Cole & Miller, 1932; Grant, 1934), though it is somewhat difficult to decide from the accounts given whether or not the ewes were actually on heat at the time of killing. There is no doubt, however, that in the present experiment when a ewe was examined while still on heat after 30 hr. from the onset of oestrus ovulation had not occurred, while if a ewe had been off heat for more than 1 hr. after this interval ovulation had occurred.

Most workers have found that ovulation had occurred when ewes were examined after the end of heat. There is considerable variation, however, in the time of ovulation noted by different workers, as, for example, the three ewes in which ovulation was actually observed at 29·1, 34·5 and 37·1 hr. after the beginning of heat by McKenzie *et al.* (1933), and the observations of Quinlan & Maré (1931), who found that ovulation occurred from the 36th to the 40th hour of heat. In the present experiment in ewes with oestrous periods of 30 hr. or more, the time of ovulation was related to the end rather than to the beginning of heat, and since the duration of oestrus varies considerably in the ewe it is thought that much of this variation in the time of ovulation must be due to differences in the length of heat.

It is quite clear that in ewes with short heat periods rupture of the follicle occurs some considerable time after the end of heat and that there is a minimum period of approximately 23–25 hr. before which ovulation does not occur, irrespective of the length of heat. There are few records by other workers of ovulation occurring in the first 24 hr. after the beginning of heat.

Quinlan & Maré's statement that the duration of oestrus appears to

depend on the time of rupture of the follicle and that delay in rupture appears to explain the prolonged oestrus occasionally observed is not substantiated. Stieve (1934) also concluded that heat persists as long as the ovary contains a mature follicle and ceases when the follicle ruptures. The ewes with short heat periods examined by the author are in contrast to this view, for oestrus had ceased several hours—in one case 7½ hr.—before ovulation occurred. Moreover, in ewe No. 46, examined 33 min. after the end of heat, the follicle had not ruptured, and in ewe No. 71, 47 min. after the end of heat, ovulation was probably just about to occur. It therefore seems that normally oestrus ceases before rupture of the follicle occurs in the ewe, as in the cow (Hammond, 1927).

SUMMARY

Thirty-one laporotomies, in addition to a number of confirmatory operations, were performed to determine the time of ovulation in the ewe. It was found that (1) ovulation did not occur during oestrus, (2) in ewes with oestrous periods lasting 30 hr. or more ovulation occurred a short time after the end of oestrus and that the time of its occurrence is related to the end and not to the beginning of oestrus, and (3) there is a minimum period of approximately 23–25 hr. before which ovulation does not occur, irrespective of the duration of oestrus.

[*Postscript*, 7 *November*. Since the above was written a further memoir has been published by McKenzie and Terrill (1937) dealing with oestrus, ovulation and the related phenomena in Hampshire, Shropshire, Southdown and Rambouillet sheep. These authors found that the time of ovulation varied from earlier than 12 hours to later than 41 hours after the onset of oestrus; further, it was found to occur as early as 11 hours before the end of oestrus and as late as 6 hours after the end of oestrus. Generally speaking, ovulation took place near the end of oestrus.

F. H. A. M.]

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the anterior mesenteric artery in every case. None of the horses on the estate have a heavy infestation according to the result of sugar flotation tests of the fæces.

Summary and Conclusions

In an outbreak of influenza and pneumonia amongst horses, an autogenous vaccine appeared to reduce the mortality rate from 50 per cent. to nil. Of the unvaccinated animals which contracted the disease, foals quickly recovered while animals in show condition succumbed in a few days. The chief after-effect of the disease was strain of the heart.

AN EXTENSION TO THE KNOWN LONGEVITY OF GAPEWORM INFECTION IN EARTHWORMS AND SNAILS

By E. L. TAYLOR, M.V.Sc., M.R.C.V.S.
Veterinary Laboratory, Ministry of Agriculture.

THE longevity of parasitic worms is a matter for the astonishment of those who have no special knowledge of the helminithological literature. Tavassor reported an observation on an infestation of *Diphyllobothrium latum* which had been carried by the human host for 40 years. Penfold reported an instance of *lænia saginata* living for 35 years, Fairley an instance of *Schistosoma* living for 30 years, and there are many other surprising reports of the great longevity of parasitic worms.

Although the intermediate stages of some species are rather short-lived, it is the rule for these to be able to await transference to the secondary host for a very considerable time. Particularly does this apply to larval forms living in invertebrates when it is unusual for there to be any marked reaction on the part of the host.

The longevity of *Syngamus* larvæ in the earthworm as shown by observations carried out at Weybridge is an interesting case in point. The continuation of observations published in the *Journal of Comparative Pathology and Therapeutics* for June, 1935, has shown that *Syngamus* larvæ may remain alive and infective in the muscles of earthworms for 4 years 4½ months; 3½

years having been the maximum period observed at the time of the previous publication.

Fig. 1 shows one of the larvæ recovered by digestion from the muscles of an earthworm and is particularly interesting on account of the thick cyst wall which surrounds it; an unusual reaction on the part of an invertebrate host.

Figs. 2 and 3 show gapeworm larvæ in the tissues of a snail, one in the salivary gland and one in a nerve ganglion. The nerve ganglia appear to be a predilection seat. In one particular snail larvæ were found in the following positions: three in the tissues of the body wall, one in the foot, one in the body cavity, one in the hemaphrodite gland, one in the liver, one in the salivary gland and 15 in the nerve ganglia of the head.

Continuation of observations reported in the above-mentioned paper showed that snails are able to carry the infection for one year one and a half months, and it is probable that that period is nowhere near the end point, which probably coincides with the longevity of the snail. According to Boycott *Helix aspersa* lives for five or six years and the common earthworms are reported to be some ten years, so that the gapeworm is well fortified against a prolonged period of the absence of suitable secondary hosts.

THE INDUCTION OF ŒSTRUS IN THE EWE

By J. ANDERSON, M.R.C.V.S.

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MUCH of our knowledge of the hormones associated with the reproductive processes has been obtained from the smaller laboratory animals and while the broad principles that have evolved from such studies are probably directly applicable to the larger domesticated animals, it is desirable that the action of the various hormone preparations should be studied in these animals themselves. It is the object of this paper to present the results of injecting œstradiol benzoate ("Dimenformon," Organon) into high-grade Merino ewes in the anœstrum experienced during lactation.

Comparatively little work has been done on the effect of injecting "œstrin" into the ewe. "Œstrin" is the term used for substances which have œstrogenic properties, i.e., substances which produce changes in the genital tract of ovariectomised rats and mice, similar to those occurring at œstrus.

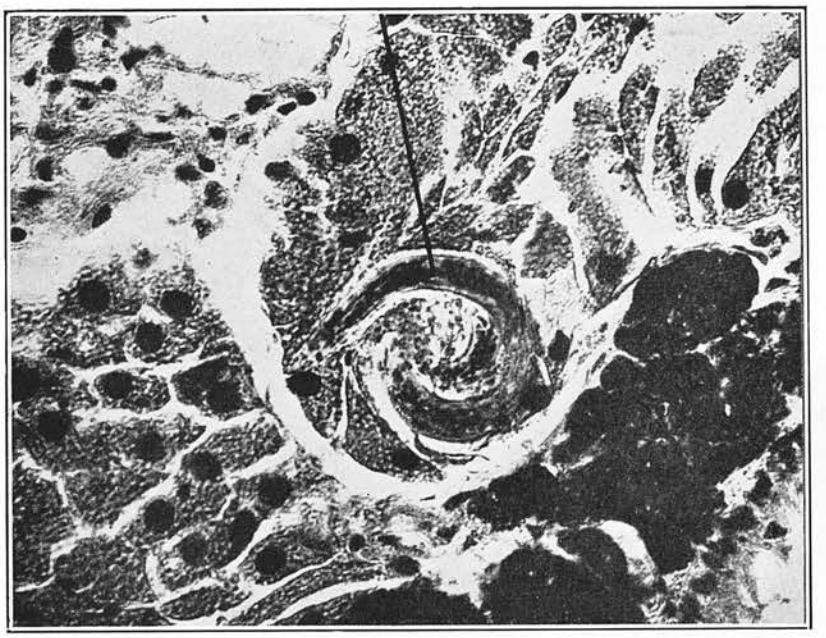

FIG. 2.

GAPEWORM LARVA IN THE SALIVARY GLAND OF A SNAIL. HELIX ASPERSA.

FIG. 3.

GAPEWORM LARVA IN THE PRINCIPAL NERVE GANGLION OF THE HEAD OF A SNAIL. HELIX ASPERSA.

ARTICLE BY E. L. TAYLOR. PAGE 327.

It is now believed, however, that the substance actually secreted by the ovary is "œstradiol." Cole and Miller (1935) successfully brought five out of seven ewes into œstrus during anœstrum by injecting œstrin. The best results were obtained with doses of 4,000-5,000 R.U. (all three ewes injected came on heat). Of two ewes injected with approximately 500 R.U. œstrin one ewe came on heat, and of two ewes injected with approximately 1,000 R.U. one came on heat. In most cases the injections were made daily on three successive days and heat occurred within three days. The ewes were killed within seven days so that the others might also have come on heat had more time been allowed. Two ewes which were injected over the period of a month (they received a total of nearly 5,000 R.U. œstrin) came on heat within two days after the last injection. Zavadovskii and co-workers (1935) found that œstrin in doses of 1,500-3,000 M.U. was ineffective in bringing ewes on heat outside the normal breeding season. Steffens (1936) obtained good results with Unden and Provetan (œstrogens). Injection of Provetan into Karakul ewes (optimum dose 200,000 I.U.) induced œstrus in 94 per cent. of cases and insemination was effective at the second or third heat period; 13 out of 16 animals receiving 100,000 M.U. of Unden came on heat. This author recommends Provetan when an autumn lambing is desired. Lesbouyries and Berthelon (1938) give the dose of œstrin for the ewe as 30,000 I.U. injected subcutaneously in one or more doses in the course of 2-3 days.

Material and Methods

It has been noted previously (unpublished data) that high-grade Merino ewes in Kenya experienced an anœstrous condition during the period of lactation. Similarly in South Africa it has been found that Merino ewes did not come on heat, when suckling, though spurious ovulation may occur (Roux, 1936). Accordingly high-grade Merino ewes which lambed in October-December, 1937, were used for this experiment. At the time of injection, December, 1937, to January, 1938, these ewes had lambed 30-73 days previously, all the injected ewes were suckling lambs and none had come on heat. 113 ewes were injected and 453 ewes which lambed at the same time as the injected ewes served as controls. 13 of these control ewes came on heat during the time of the experiment; four of these were suckling and nine were dry. The remainder of the control ewes began to come on heat towards the end of March, i.e., four to five months after lambing. It is therefore clear that these grade Merino ewes which are suckling lambs can be safely used to test the effect of an œstrus-inducing preparation since during lactation only a very small number experience heat spontaneously and this occurs mostly in dry ewes.

Œstradiol benzoate ("Dimenformon," Organon) in the form of an oily solution was injected intramuscularly in the thigh. This solution is said to

contain 50,000 International Benzoate Units per 1 c.c. Appropriate dilutions were made with olive oil for testing different concentrations of the hormone.

Results

Of the 113 ewes injected with œstradiol benzoate 84 (74.3 per cent.) came on heat within 21 days (Table I). The majority of these came on heat in 1-5 days following injection. The next most frequent interval before heat occurred following injection was 16-21 days. Thirteen other ewes came on heat in 22-40 days following injection, but these ewes have been excluded from the results on account of the long interval between injection and heat.

A single injection of 2,500 units was just as effective (five out of seven ewes responded) as single injections of 25,000 units (three out of five ewes responded) and 50,000 units (two out of three ewes responded). There does not seem to be any difference in giving the injections in three doses once daily, or in three doses, a.m., p.m., and a.m. (see results of injecting 3,000 and 7,500 I.U.). 1,000 units in five doses (twice daily for three days and on the morning of the third day) gave slightly better results (100 per cent.) than 5,000 units in one dose (80 per cent.). The maximum response was obtained with doses of 5,000-6,000 units.

An interesting result was that the induction of œstrus by this means started the œstrus cycle in a large number (73 of the 84 ewes that came on heat within 21 days) of the injected ewes. It was noted that with successive cycles the length of the interval between the heat periods tended to approximate more and more to the normal length of the dicestrous interval. Thus the length of the interval between the first and second heat period was 26 days, between the second and third 19 days, betwen the third and fourth 18 days, and between the fourth and fifth 17 days.

The ovaries of a number of injected and non-injected ewes were examined by laparotomy using a local anæsthetic (Table 2). Four non-injected ewes were examined at different periods following lambing; two ewes examined on the 42nd day after lambing showed no corpora lutea and one of these ewes examined on the 55th day after lambing had still not ovulated. One ewe on the 59th day after lambing had two large follicles in one ovary but no corpus luteum and had therefore not ovulated and one ewe that died on the 82nd day after lambing had a whitish old corpus luteum in the ovary. 12-15 injected ewes were operated on before they received œstradiol benzoate. Nine of these ewes showed in their ovaries follicles of various sizes, but no corpora lutea. Two ewes, both 53 days after lambing, had a whitish old corpus luteum in their ovaries and one ewe 35 days after lambing had a small red corpus luteum. Roux's observation that spurious ovulation can occur in the absence of heat during lactation in the Merino is therefore confirmed. It is clear also that follicular growth and regression occurs in the Merino during lactation. The ovaries of seven ewes were examined a few days after the first

induced heat period (No. 123 had not come on heat when she died two days after injection) and ovulation had occurred in two ewes, No. 512 and No. 416 (in the former heat occurred two days after injection and in the latter 16 days). The ovaries of five ewes were examined after the second heat period, i.e., after the heat period which followed spontaneously after the induced heat period). Two of these ewes had recently former corpora lutea and had therefore ovulated at the second heat period. Two of these ewes which had not ovulated at the second heat period did so at the third.

It is clear therefore that œstradiol benzoate (1) can induce œstrus in anœstrus ewes, (2) is capable of starting the œstrous cycle in grade Merino ewes during the lactation period in Kenya, and (3) once heat has been induced it appears that ovulation probably occurs at the second or subsequent heat periods.

Discussion

The majority of the injected ewes that came on heat within 21 days did so within the first five days. It is perhaps a debatable point whether those which came on heat later than five days following injection did so as a result of the injection of œstradiol benzoate. It appears reasonable, however, to suppose that the reproductive system was stimulated as a result of the injection since the control ewes, except for isolated cases, did not experience œstrus at this time. Moreover, it may be significant that there are two peaks of response in relation to time following injection, namely, a few days after the injection and again 16-21 days after the injection, which corresponds roughly to the normal cyclical interval in the ewe. A similar argument might also be applied to those ewes which came on heat 22-40 days after the injection, but for the present this must remain an open question since the interval is rather long and also the time at which some of these ewes at least came on heat was approaching the time when the control ewes began to come on heat.

The question of dosage requires further investigation, but it would seem that the optimum dose is probably in the neighbourhood of 5,000 International Units of œstradiol benzoate and that best results would be obtained by giving this amount in divided doses.

Possibly the most practical point that has emerged from these experiments is that one injection (or a series of injections within a few days) can initiate not merely an isolated œstrus but the full sexual cycle. Within the time limits of the experiment it was found that a considerable number of ewes experienced a second œstrus and some as many as five. It is of interest to note that Steinach, Staheli and Gruter (1934) have found that in sterile anœstrous cows and in pigs a single injection of dihydro-follicular hormone benzoate (50,000 units for cows and 25,000 units for pigs) initiated the full sexual cycle and not merely an isolated œstrous. Likewise Steffens (1936) has found

that a single injection of Provetan has made conception possible at the second or third heat periods in the ewe.

The question now arises as the possible effect that œstradiol benzoate may have indirectly on ovarian function and particularly on ovulation. In two ewes in this experiment examined a few days after the first heat period following injection of œstradiol benzoate newly-formed corpora lutea were found, indicating that ovulation had occurred. It cannot be overlooked, however, that ovulation in these ewes might have been spontaneous and not due to the action of the hormone since ovulation can occur spontaneously in anœstrous ewes during lactation. Cole and Miller also noted the presence of corpora lutea in the ovaries of ewes receiving œstrin. They concluded that ovulation in these instances was spontaneous and no doubt occurred before the injection of œstrin and independently of it.

The effect of administration of œstrin on the anterior pituitary has been investigated by numerous workers. Castration changes in the pituitary have been prevented by appropriate doses of œstrin (Hohlweg and Dorhm, 1931, 1932) and by ovarian transplants into adult castrated rats (Haterius and Nelson, 1932). It is thought that œstrin increases the secretion of a luteinising hormone by the pituitary (Hohlweg, 1934, Lane, 1935, Wolf, 1935). Lane concluded that the effects of œstrin on the production of gonadotropic hormone(s) by the pituitary occurred in two stages: (1) the early effect was an increased liberation of gonadotropic hormone(s), (2) then occurred a period of diminished liberation and secretion of follicle stimulating hormone and in this stage there was an increased secretion and liberation of luteinising hormone.

It would seem that the injection of œstradiol benzoate into the ewe during the anœstrum of lactation brings about the resumption of the normal œstrous cycle through stimulation of the gonadotropic activities of the pituitary. The delay in the occurrence of heat for 16-21 days in a number of ewes following injection of the œstradiol benzoate would seem to indicate that this hormone has had an effect on the anterior pituitary similar to that which is probably exerted by the œstrus-inducing hormone secreted by the ovaries themselves during the normal œstrus cycle in the ewe.

Summary

The intramuscular injection of œstradiol benzoate induced heat in 84 (74.3 per cent.) of 113 grade Merino ewes during the anœstrous period of lactation. The optimum dose was about 5,000-6,000 I.U. This hormone preparation induced not merely a single heat period but caused the resumption of the normal œstrous cycle probably through its effect on the gonadotropic activity of the anterior pituitary.

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TABLE I

INJECTION OF OESTRADIOL BENZOATE INTO EWES

No. of Ewes	*Dose of O.B. in I.U.*	*Interval in Days between Injection and first Heat*				*Ewes that came on Heat*	
		1-5	6-10	11-15	16-21	*No.*	*%*
7	2,500 x 1 = 2,500	2	1	1	1	5	70
10	1,000 x 3 = 3,000 once daily	3	1	—	2	6	60
10	1,000 x 3 = 3,000 a.m., p.m., a.m.	4	—	—	1	5	50
20	5,000 x 1 = 5,000	11	1	—	4	16	80
8	1,000 x 5 = 5,000	8	—	—	—	8	100
10	1,000 x 6 = 6,000 twice daily	7	—	1	2	10	100
20	2,500 x 3 = 7,500 twice daily	6 7	—	2	4	12	60
10	2,500 x 3 = 7,500 a.m., p.m., a.m.		—	—	1	8	80
10	2,500 x 6 = 15,000 twice daily	7	—	—	2	9	90
5	25,000 x 1 = 25,000	—	—	1	2	3	60
3	50,000 x 1 = 50,000	2	—	—	—	2	66

Dose—O.B. in I.U. = Œstradiol Benzoate in International Units.

TABLE II. EXAMINATION OF OVARIES OF INJECTED EWES

No. of Ewe	Lambed	Ovaries Examined	Injected Dose	1st Heat	Ovaries Examined	2nd Heat	Ovaries Examined	3rd Heat	Ovaries Examined
640	3/11/37	—	7/12/37 25,000x1	26/12/37	—	11/1/38	13/1/38 Ovulated		
659	28/10/37	—	7/12/37 25,000x1	26/12/37	—	30/1/38	2/2/38 Ovulated		
512	18/11/37	—	11/ 1/38 2,500x3	13/ 1/38	19/1/38 Ovulated	—	—		
305	16/10/37	8/12/37 1 large follicle in L.O.	13/12/37 2,500x1	28/12/37	13/1/38 Not ovulated	14/1/38	19/1/38 Not ovulated	12/2/38	19/2/38 Ovulated
472	30/10/37	9/12/37 2 large follicles in R.O.	13/12/37 2,500x1	31/12/37	13/1/38 Not ovulated	15/1/38	19/1/38 Not ovulated	—	
196	5/11/37	8/12/37 Small follicles	13/12/37 2,500x1	22/12/37	—	25/1/38	27/1/38 Not ovulated	12/2/38	15/2/38 Ovulated
296	12/11/37	11/1/38 No large follicles	11/ 1/38 2,500x3	28/ 1/38	2/2/38 Not ovulated				
169	1/12/37	11/1/38 1 small red c.l. in L.O.	11/ 1/38 2,500x3	—		—			
L8	27/11/37	11/1/38 1 large follicle in L.O.	11/ 1/38 2,500	14/ 1/38	19/1/38 Not ovulated				
302	26/11/37	11/1/38 Small follicles	11/ 1/38 2,500x3	—		—			
416	13/11/37	12/1/38 Old c.l. in R.O. 1 large follicle in L.O.	12/ 1/38 2,500x3	28/ 1/38	9/2/38 Ovulated	—			
27	21/11/37	12/1/38 No c.l. or follicles	12/ 1/38 2,500xs	—					
84	13/11/37	12/1/38 1 old c.l.	12/ 1/38 2,500x3	—					
340	23/11/37	12/1/38 No c.l. or large follicles	12/ 1/38 2,500x3	—					
123	14/11/37	12/1/38 2 large follicles in R.O.	12/ 1/38 2,500x3	—	Died 14/1/38				

R.O.—right ovary; L.O.—left ovary; c.l.—corpus luteum. Dose—International Units of Oestradiol Benzoate.

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FURTHER INVESTIGATIONS ON THE SEMEN OF THE BULL

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FURTHER INVESTIGATIONS ON THE SEMEN OF THE BULL

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It is the object of this paper to present and discuss the results of investigations on the semen of the bull, in continuation and extension of work previously reported (Anderson, 1939, 1940). In particular, a more detailed examination of the semen of bulls of known fertility and of bulls in the early stages of epididymitis has been made.

Material and Methods

The semen was collected, as in artificial insemination, with the artificial vagina, following the method of Walton and Russian workers. This method has already been described in full (Anderson, 1938, 1940). For storage, semen was placed in pyrex test tubes, the tube filled with liquid paraffin (Walton, 1936), corked and sealed with candle wax. It was then wrapped in cotton wool and placed in a thermos flask at a temperature of 7° to 10° C. The maintenance of the temperature in the flask was best accomplished by keeping the flask in a refrigerator.

The general classification of the bulls investigated is given in Table I. For convenience, bulls classed under sperm " good " are subdivided into (*a*) E.S.—Experimental Station bulls, (*b*) Farm A bulls and (*c*) Other bulls. The bulls on the Experimental Station were pure-breds except for one grade Ayrshire (Ag). Those on Farm A were Friesians, mostly pure-breds. The other Experimental Station bulls are designated thus : H = Hereford, Su = Sussex, AA = Aberdeen-Angus, A2 = Ayrshire and F = Friesian.

TABLE I

SUMMARY OF BULLS, 1938-1939

Classification	Sperm tests	Clinical examination only	Total
Clinically normal			
Sperm good ...	51	—	51
Sperm poor ...	16	—	16
Sperm not examined ...	—	6	6
Clinically abnormal			
Epididymitis ...	9	8	17
Testicle abnormal	5	2	7
Total ...	81	16	97

SEMEN EXAMINATION

Volume.—The volume of ejaculates in the different classes of bulls is shown in Table II (*over-leaf*). In general there was a tendency for the mean volume to decrease from normal to abnormal bulls, but there was no hard and fast line between the different classes. Much of the variation was no doubt due to individual differences and possibly age, management and feeding. It has already been noted that the ejaculate of young bulls is smaller than that of adult bulls. The ejaculate in cases of epididymitis is often as large as that from fertile bulls and one bull (Sk.) with bilateral enlargement of the testes, had on the average, an ejaculate of normal size, though the variation in size of the ejaculate was greater than is usually found (Table II).

Spermatozoa occupy but a small part of a total ejaculate, the bulk of which is formed by the accessory secretions. Therefore the volume of the ejaculate may be regarded more as an indication of secretory activity of the accessory glands than of the testes. The degree of involvement of the accessory glands in abnormal conditions may be suspected from the size of the ejaculate. The primary cause of absence of spermatozoa from the ejaculate in cases of epididymitis is occlusion of the epididymal ducts. This can occur without causing much change in the volume of the ejaculate, *e.g.*, three bulls with epididymitis that were still ejaculating small quantities of spermatozoa, had an average ejaculate of 3·3 mls. while six other bulls also with epididymitis, but which ejaculated no spermatozoa, had an average volume of 2·9 mls.

The three Experimental Station bulls of the beef type, Hereford, Sussex and Aberdeen-Angus had smaller ejaculates than the dairy types. Indications of possible breed differences have already been noted. The mean volume of the ejaculates of five of these bulls was very similar in the two periods, 1935-37 and 1938-39 ; there was a slight decrease in the mean volume of the ejaculates of all bulls with the exception of A2. The grade Ayrshire bull (Ag) had the largest mean volume of ejaculate in both periods and a Shorthorn grade bull (S1) had the next largest average ejaculate (5·94 mls.) in the period 1935-37.

Seasonal changes in the volume of ejaculates of those bulls (Ag, A2, H, Su and AA1) which performed most services during 1938 and 1939 are shown in Table VII and Fig. 1. The data in Fig. 1 are smoothed by the formula $\frac{a + 2b + c}{4}$ (where " b " is the particular month and " a " and " c " the preceding and following months). These data are presented without comment, since investigations of seasonal changes would require more controlled conditions than was possible or attempted in the period under review.

TABLE II
Volume of Ejaculates

Classification	Number of Bulls	Number of Ejacula	Volume in mls. Mean	S.D.
Clinically normal				
Sperm good (*a*) E.S.	6	241	4·40 ± 0·13	2·1
(*b*) A	10	27	3·8	
(*c*) Others	30	60	3·22 ± 0·20	1·5
Sperm poor	16	27	3·2	
Clinically abnormal				
Epididymitis	9	15	3·2	
Testicle abnormal	1 (SK)	51	5·45 ± 0·45	3·2
	4	9	2·7	

Bull	1938-1939 Experimental Station Number of Ejacula	Mean	S.D.	1935-1937 Number of Ejacula	Mean	S.D.
H	72	3·65 ± 0·20	1·71	9	3·7	
Sul	36	3·24 ± 0·30	1·75	10	3·7	
AA	49	3·65 ± 0·27	1·89	9	5·8	
Ag	39	5·81 ± 0·37	2·32	25	6·77 ± 0·37	1·3
A2	35	5·66 ± 0·31	1·87	18	5·4	
F	10	6·05	—	—	—	

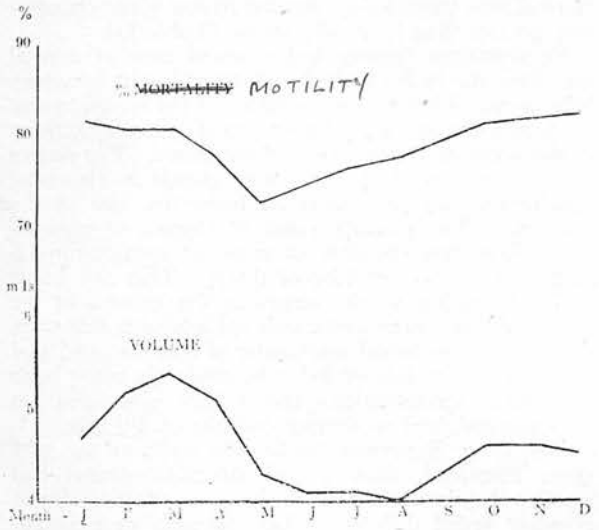

FIG. 1—Seasonal Variation in Motility and Volume of Ejaculates.

Density of Spermatozoa.—The concentration per c.mm. and the total number of spermatozoa per ejaculate are shown in Table III. Spermatozoa were absent from eleven ejaculates from six bulls with epididymitis and three other bulls with epididymitis had ejaculates containing an average of 227,000 spermatozoa per c.mm. Counts were made on five bulls with testicular abnormalities ; of these, one, with a very small right testis, had an average of 132,000 spermatozoa per c.mm. in five specimens, and another (SK) which had bilateral enlargement of both testes had an average of 539,000 spermatozoa per c.mm. For the 53 specimens from 29 bulls with " good " sperm, the number of spermatozoa in thousands was 748,000 ± 52,500 (s = 395,000). Three (5·3 per cent.) specimens had a count below 250,000 spermatozoa per c.mm. and 15 specimens (26·3 per cent.) below 500,000 per c.mm.

For 29 bulls with sperm " good," the mean total number of spermatozoa per ejaculate in thousand millions was 4·04 ± 0·14 (s = 2·79). In the three bulls with epididymitis that ejaculated spermatozoa the total count was 0·683 per ejaculate ; for all bulls with epididymitis, however, the average was 0·146 per

TABLE III
Concentration of Spermatozoa

Classification	Number of Bulls	Spermatozoa Number per c.mm. in thousands Mean	Range	Percentage abnormal
Clinically normal				
Sperm good (*a*) E.S.	6	944	320-1, 227 (18)	10·5 (10)
(*b*) A	10	716	60-1, 133 (26)	6·9 (19)
(*c*) Others	13	705	430-1, 530 (13)	10·6 (22)
Sperm poor	16	512	50-1, 270 (13)	16·3 (8)
Clinically abnormal				
Epididymitis	9	49	0-340 (14)	55·5 (2)
Testicle abnormal	5	370	110-580 (12)	25·0 (7)

Figures in parenthesis indicate number of ejaculates.

ejaculate. In the bull with the very small right testis the total count was 0·324 and in the bull (Sk) with bilateral enlargement of the testes it was 3,261 thousand millions per ejaculate. Individual results on the concentration of spermatozoa per c.mm. and the total number per ejaculate are too few to detail here; they are mentioned later in connection with fertility.

Motility.—Particulars of the motility of the ejaculates of the different classes of bulls are shown in Table IV (10 = 100 per cent. activity, 9 = 90 per cent. activity, etc.). Since 70 per cent. motility is taken as the permissible limit for artificial insemination, it is of interest to note the number of specimens which showed 70 per cent. or greater motility. In the three classes of bulls, Experimental Station, Farm A and " others," on which the bulls produced " good " sperm there were 88·5, 66·7 and 76·6 per cent. of ejaculates respectively with this motility. For bulls with sperm " poor " the figure was 14·7 per cent.; for bulls with atrophy of the testes 25·0 per cent., and for hypertrophy of the testes 6·7 per cent., and for cases of epididymitis, none.

Abnormal Spermatozoa.—A few estimations of abnormal types of spermatozoa in the Experimental Station bulls gave an average of 10·5 per cent. with a range of from 4 to 16 per cent. For Farm A bulls, the average was 6·5 per cent. with a range of from 3 to 10 per cent. For other clinically normal bulls with " good " sperm the average was 10·6 per cent. and the range 4 to 20 per cent. The 16 clinically normal bulls with poor sperm had, with the exception of one bull, either poor motility or small concentration of spermatozoa or both. This one bull had good motility (80 per cent.), but 44 per cent. of spermatozoa were abnormal and the number of spermatozoa per c.mm. (490,000) was also rather low. Two cases of epididymitis had 25 per cent. and 86 per cent. of abnormal spermatozoa. Two bulls with both testes which are shown in Table VII and Fig. 1. It is of interest to note, however, that of 22 sperm specimens of poor motility collected during 1938 and 1939 from the Experimental Station bulls, 14 were collected during the months of May, June and July.

Table IV—Motility

Classification	Number of Bulls	Number of Specimens	Motility 10	9	8	7	6	5	4	3	2	1	N	A	Mean Motility
Clinically normal															
Sperm good (*a*) E.S.	6	254	10	76	121	18	9	4	3	3	6		3	1	78
(*b*) A	10	27	1	7	4	6	4	4				1			69
(*c*) Others ...	30	52	2	17	13	8	6	4	1	1					76
Sperm poor	16	27			3	1	2	6	4	3	2	3	3		39
Clinically abnormal															
Epididymitis	9	14										3		11	
Hypertrophy of testis	1 (SK)	51				4	4	5	11	5	9	9	4		37
Atrophy	1	5					2		2	1					
	2	3				2							1		48

Bull	Number of Specimens	Experimental Station Motility 10	9	8	7	6	5	4	3	2	1	N	A	Motility Mean	70 per cent. and over
H	73		29	36	4	2	2							82	86
A2	41	4	14	13	2	2				5			1	75	82
Ag	42	2	11	21	3		1	1	1			2		76	83
AA1	51	1	6	30	8	2	1	1	1	1				76	80
Su1	36	2	8	19	1	3		1	1			1		77	84
F	11	1	8	2										89	89
Total	254	10	76	121	18	9	4	3	3	6		3	1	78	89

N = No motility; A = spermatozoa absent.

The motility figures for individual bulls on the Experimental Station and Farm "A" are for all the ejaculates from these bulls for the period 1938-39. Occasionally, motility on the first ejaculation was poorer than on the second or third ejaculations, but there were few occasions on which second ejaculations had to be collected because of poor quality of the first ejaculation (Table V). The figures given may therefore be taken as representative of the motility of sperm of the different bulls. In bulls classed under sperm " poor," eight bulls on a single ejaculation gave sperm of poor motility; had a second collection been obtained from these bulls, it is possible that ejaculates showing better motility might have been obtained.

The remarks made about seasonal variation and volume of ejaculates apply also to motility, data for enlarged had 19 per cent. (bull Sk.) and 30 per cent. respectively. The mean percentage of abnormal spermatozoa for all testicular abnormalities detected was 25 per cent. Clinical changes in the testes and epididymes were therefore in most cases associated with an increase in the percentage of abnormal spermatozoa, which agrees with previous observations.

Frequency of Collection

The effect of the frequency of collection can be examined in two groups, (*a*) in which there was an interval of minutes between collections of sperm and (*b*) in which there was an interval of days.

Most workers have observed that the first ejaculation in normal fertile bulls may be of inferior quality to subsequent ejaculations. A comparison between first,

second and third ejaculations is shown in Table V. In clinically normal bulls, the volume and motility improved on the second jump. Even in bulls with sperm "poor" there was some improvement in motility on the second ejaculation. In the bull with bilateral hypertrophy (Sk.) of the testes, there was a decrease in volume from the first to the third ejaculation; while the motility, which was poor in all jumps, increased slightly on the second ejaculation but was practically absent in the third.

On 24 occasions double jumps were obtained from the Experimental Station bulls during 1938 and 1939 (Table V). On four of these double jumps the motility was good enough (70 per cent.) on the first collection to use the sperm for artificial insemination. Therefore, on only 20 occasions was it necessary to collect a second specimen of sperm because of poor quality of the first ejaculate, *i.e.*, in about 8 per cent. of ejaculates. In four specimens from different bulls, the volume and motility were less on the second jump, but in most cases they were better.

The number of days between ejaculations had little or no effect on either motility or volume of the ejaculate (Table VI). There was a slight decrease in motility and increase in volume as the interval increased from 1 to 20 days, but this tendency was not maintained for longer intervals. At the first ejaculation after quite lengthy intervals the volume and motility have been good. In one bull, for example (Ag) the first collection after an interval of seven months was 80 per cent. motile.

It is a plausible theory that a too lengthy interval between ejaculations is responsible for poor quality of sperm in the subsequent ejaculation, on the grounds that spermatozoa are continually being produced, which if not ejaculated, undergo degeneration and are eliminated at the next ejaculation. In the present data there is no relationship between poor quality of sperm and interval since last collection of sperm or frequency of collections.

Table VI

Frequency of Ejaculation

Interval in days between Ejaculations	Number of Ejaculations	Mean per cent. Motility	Mean Volume in mls.
1 to 5	75	83	4·6
6 to 10	64	79	4·5
11 to 15	26	76	4·9
16 to 20	13	74	5·5
21 to 25	5	80	5·5
26 to 30	9	72	5·3
31 to 35	1	90	7·0
36 to 40	1	30	5·0
41 to 45	3	80	6·0
46 to 50	1	90	5·0
51 to 55	—	—	—
56 to 60	3	80	4·7

Table VII

Monthly Variation in Ejaculates

	Number of Ejaculations	Mean per cent. Motility	Mean Volume in mls.
January	9	82	4·4
February ...	12	80	5·3
March	16	82	5·6
April	19	80	5·3
May	18	69	4·1
June	28	76	3·8
July	24	78	4·5
August	22	75	3·7
September ...	23	84	4·2
October ...	13	80	4·9
November ...	21	84	4·4
December ...	11	82	4·6

Table V

Comparison Between First and Subsequent Ejaculates

Classification	Number of Bulls	Number of Repeated Ejaculates	Volume in mls. 1st	Volume in mls. 2nd	Volume in mls. 3rd	Motility 1st	Motility 2nd	Motility 3rd
Clinically normal								
Sperm good (*a*) E.S.	6	24	3·3	4·0	—	53	75	—
(*b*) Others	8	8	2·8	3·7	2·7	69	80	84
Sperm poor	7	7	2·4	2·4	—	37	50	—
Testicular abnormality	1 (SK)	19	6·9	5·7	1·2	25	39	6
Epididymitis	4	4	2·1	2·1	2·8	R	R	N

Experimental Station

Bull	Total number of Ejaculates	Number of Repeated Ejaculates	Volume in mls. 1st	Volume in mls. 2nd	Motility 1st	Motility 2nd
H	85	8	3·1	3·8	70	80
Su1	59	3	2·2	3·0	30	90
AA1	61	3	4·0	5·5	60	70
F	11	1	7·0	7·0	90	90
A2	71	6	4·1	5·4	30	60
Ag	74	3	4·6	6·0	40	60
		24	3·3	4·0	53	75

Clinically Normal Bulls

The general characteristics of sperm from the different classes of bulls is summarised in Table VIII. In bulls with "good" sperm the figures for volume, motility, concentration and total number of spermatozoa and percentage of abnormal spermatozoa are such as are associated with fertility. Some of the Experimental Station bulls have been under observation for over four years and have consistently produced sperm of high quality during this time.

Table VIII—General

Classification	Mean volume of Ejaculate in mls.	Mean per cent. Motility	Mean number of Spermatozoa in thousands per c.mm.	Mean per cent. abnormal Spermatozoa
Clinically normal				
Sperm good (*a*) E.S.	4·40	78	944	10·5
(*b*) A	3·80	69	716	6·9
(*c*) Others	3·22	76	705	10·6
Sperm poor	3·20	39	512	16·3
Clinically abnormal				
Epididymitis	3·20	—	49	55·5
Testicular hypertrophy	2·40	32	539	23·0
atrophy	2·84	48	132	29·2

In bulls with "poor" sperm, on the average, motility, concentration and total number of spermatozoa were reduced and the percentage of abnormal spermatozoa was increased. In 11 of the 16 bulls in this class the motility was less than normal. In one bull there was a high percentage of abnormal spermatozoa (44) and the concentration and total number of spermatozoa (490,000 per c.mm. and 245 millions respectively) were below normal; the motility on two consecutive collections was 50 per cent. and 80 per cent. This was a young bull which had had very few services. In four bulls there was both poor motility and a concentration of less than 300,000 spermatozoa per c.mm. It is clear that an ejaculate may be normal in respect of, say, motility and below normal for other criteria such as concentration of spermatozoa and number of abnormal spermatozoa.

Clinically Abnormal Bulls

In most cases of epididymitis there was a complete absence of spermatozoa, but when they were present in the ejaculate they were few in number, of poor motility and many abnormal forms were observed. Similar observations have been reported previously (Anderson, 1939, 1940).

One natural question that arises is the effect of unilateral epididymitis on the sperm. Of a total of 12 bulls with unilateral epididymitis observed from 1936 to 1939, seven ejaculated no spermatozoa and the ejaculates of the five other bulls contained few spermatozoa which exhibited no motility or feeble motility. The ejaculate of one of these five bulls contained 240,000 spermatozoa per c.mm. and 25 per cent. abnormal spermatozoa. The sperm picture was thus the same in unilateral as in bilateral epididymitis. It is obvious that functional disturbances must arise before gross clinical changes can be detected. This is supported by five cases observed during the course of the disease. Two of these bulls were tested during 1936 and 1937 and both had azoospermia before clinical signs of epididymitis were found.

One case of epididymitis was observed in the initial stages. This bull (S), a pure-bred Friesian, aged 22 months, had only three natural services between October 10th and November 2nd, 1937, the only services since the bull arrived in Kenya. Two of these services were fertile. On January 1st, 1938, no spermatozoa were present in his ejaculate which contained thick, yellow, stringy mucus. On four other occasions up to March 10th he gave similar ejaculates. On February 22nd no clinical evidence of epididymitis could be detected, but on April 20th he had marked epididymitis. The history of this bull is of particular interest, as one of the cows (x) served by him on October 25th, which failed to conceive, had been served previously on September 18th and October 9th by another bull (T) which was found to have epididymitis when examined on November 4th. This same cow (x) was served later, in January and February, 1938 by a grade bull (U) which subsequently developed epididymitis. It is as a rule difficult to obtain a clear history of the course of infection in a herd and the author is therefore particularly indebted to Mr. Byng-Hall, of Soysambu Estate, for the details of this case. It appears that the cow (x) was infected by bull (T) at service on September 18th or October 9th and then passed on the infection to bull (S) on October 25th and to bull (U) in January or February, 1938. The cow was thus in a known infective condition for about four months from September to January. The bull (S) developed clinical signs of epididymitis in 58 days between February 22nd and April 20th; such signs might have been detected at an earlier date, if the bull had been examined. There was a period of 82 days between the probable date of infection and the date on which his sperm was first examined and found to be poor. It is of interest to note that this bull successfully impregnated a cow eight days after contracting infection.

In May, 1938, the ejaculate of a pure-bred Ayrshire bull contained only 100,000 spermatozoa per c.mm., which were sluggishly active, and in which there was a high percentage of abnormal spermatozoa. The ejaculate was creamy in consistency but on dilution it showed floccules. Many large epithelial cells were present. Clinically, the epididymes were normal on this date, but by August, 1938, there was marked epididymitis on both sides. The author is indebted to Mr. Beaumont, M.R.C.V.S. for particulars of another

bull which gave poor sperm when first tested and which about six weeks later showed the first signs of epididymitis.

From a consideration of these cases it is clear that sperm production is seriously affected before any clinical evidence of the disease can be detected. From clinical evidence with infrequent examinations it is not possible to say how long it takes for sperm to become altered after infection, how quickly clinical symptoms develop, or how long sperm may be altered before clinical symptoms develop. No doubt there is some variation according to severity of infection and individuality of the bulls. The most exact information available so far is from bull S, which ejaculated poor sperm and showed clinical symptoms 82 days and 177 days respectively after the date of probable infection. The sperm was poor for a period of 95 days before the bull was found to be clinically affected. Clinical symptoms may develop in a short time as, for example, in Mr. Beaumont's case in which this apparently happened in a few weeks.

Testis.—During the whole period 1936 to 1939 there were eight bulls in which definite clinical abnormalities were observed in the testes. In four of these bulls the testes were enlarged. From two bulls with unilateral enlargement of the testes, the ejaculates contained (*a*) no spermatozoa and (*b*) spermatozoa of poor motility (10 to 20 per cent.). Two other bulls with both testes enlarged produced ejaculates of poor motility; the ejaculate of one of these bulls contained few spermatozoa, of which a high percentage were abnormal and the other bull (Sk.) produced poor sperm over a period of nearly three years.

Of three bulls with unilateral atrophy of the testis, two produced dead sperm, one with 52 per cent. abnormal types, and the ejaculate of the other bull was of poor motility and contained a low concentration of spermatozoa, 132,000 per c.mm. Another bull with both testes small, ejaculated no spermatozoa. A further two bulls in which one testis was thought to be rather small ejaculated sperm of normal motility, though one had 20 per cent. abnormal spermatozoa. It may therefore be concluded that, when the testes appear to be markedly abnormal on clinical examination, it is likely that the sperm is also abnormal.

Character of Semen and Fertility

Sperm tests were made on a number of bulls whose breeding histories at the time of the tests were known. It is convenient to consider these bulls in two groups (*a*) those for which one or more sperm tests were made during a period for which their actual fertility is known, and (*b*) those whose sperm was used solely for artificial insemination and for which the characters of every ejaculate used were known.

Group A. *Sterile Bulls.*—The 15 bulls with clinical epididymitis present no difficulties for none of them ejaculated spermatozoa. The development of this condition has already been discussed. The quality of the sperm becomes affected some time before clinical symptoms develop and it is therefore highly probable that a bull becomes sterile before the disease is clinically evident. On the other hand, a bull may be fertile for a certain period after infection, as bull S shows.

The Ayrshire bull SK. (particulars of sperm are given earlier in this paper) which has both testes enlarged, was sterile at the time of the first test nearly three years ago. It has produced poor sperm ever since and although its fertility has not been tested during this period, it is considered extremely unlikely that it was capable of getting, at the most, more than a very small percentage of calves. The conjunction of poor sperm and clinical symptoms gave this bull a poor prognosis, when first examined, which has not altered after a long period of observation and testing.

A single collection from one bull (80) in June, 1938, was 80 per cent. actively motile when collected, but was dead after storage for 24 hours. The ejaculate contained 320,000 spermatozoa per c.mm. and a total of 1,312 million spermatozoa. The percentage of abnormal spermatozoa was 13·0. From March to July, 1938, this bull had eleven services of which none was fertile, but in August two out of four services were fertile. The only comment that can be made on this bull is that the number of spermatozoa in the ejaculate was below normal.

Fertile Bulls.—The sperm of the eleven fertile bulls (bulls on Farm A) showed on the average a high density of spermatozoa with good motility and a low percentage of abnormal spermatozoa (Table IX). A few ejaculates from these bulls were of small volume, poor motility and contained a small number of spermatozoa. These were all from single jumps and second ejaculates could not be obtained because the bulls were unwilling to ejaculate again. The low figures in the range for number of spermatozoa per c.mm. and total number of spermatozoa per ejaculate may therefore not be quite typical of the bulls. Because a fertile bull may ejaculate sperm of poor quality on the first collection, the necessity for obtaining two or more ejaculates when the first is bad, is emphasised. No differences could be detected in the sperm to account for the variation in the fertility of these bulls.

Group B.—The data in this group refer to the Experimental Station bulls which were used solely for artificial insemination for the period January, 1936, to August, 1939. The calving percentage for this period, when artificial insemination was the sole breeding method, was 85 per cent. The number of inseminations required for conception varied from 1·18 (bull F) to 2·71 (bull A3), with an average of 1·79 (Anderson, 1941).

The estimation of the quality of the sperm was, in the majority of cases, based on motility and density, and from time to time, haemocytometer counts were made to determine the exact number of spermatozoa per c.mm. and the total number of spermatozoa per ejaculate, and counts were also made of the number of abnormal spermatozoa. The general features of the ejaculates, according to the number of services required per conception, are summarised in Table IX; these data are given in more detail earlier in the paper. The general features of the ejaculates of these bulls were: volume 4 to 5 mls., motility 80 to 90 per cent; number of spermatozoa per c.mm. 800 to 900 thousand; total number of spermatozoa per ejaculate about 5,000 million and the percentage of abnormal spermatozoa about 10 per cent.

Within the above range of fertility none of the criteria used was of much value for indicating differences in fertility. The data for the two bulls with 1·18 and 2·71 services per conception are, however, few in number and examination of the two other groups with 1·5 to 2·0 and 2·1 to 2·5 services per conception shows certain minor differences (Table IX), but apart

Table IX

Character of Semen and Fertility

Group A	Services per Conception 1·51-2·00	2·01-2·50	2·51-3·00	3·01-3·50
Number of bulls	3	4	3	1
Number of services	28	174	21	31
Number of ejaculates	6	18	7	5
Volume, mean, in mls.	3·9	3·9	3·9	4·5
Percentage motility on collection	60	75	73	74
Percentage motility after 24 hours	36	13	20	17
Percentage of ejaculates with motility below 70 per cent. on collection	22	26	43	20
Concentration of spermatozoa. Number in thousands per c.mm. :				
(*a*) mean	667	656	893	460
(*b*) range	330–1140	490–1130	390–1180	290–640
Total number in ejaculate in millions :—				
(*a*) mean	2920	2448	3570	1683
(*b*) range	759–6786	1100–6650	616–9126	870–2496
Percentage abnormal spermatozoa	6·3	8·0	6·0	9·0

Group B	1·01-1·50	1·51-2·00	2·01-2·50	2·51-3·00
Number of bulls	1	3	3	1
Number of services	20	336	127	19
Number of ejaculates	11	258	204	11
Volume, mean, in mls.	6·05	4·32	5·04	5·50
Percentage motility, mean	90	87	80	93
Percentage ejaculates below 70 per cent. motility	0	5·4	10·3	0
Mean percentage motility of sperm stored for				
(*a*) 24 hours	68 (8)	72 (46)	66 (54)	—
(*b*) 48 ,,	55	56	43	
(*c*) 72 ,,	34	30	22	
Concentration of spermatozoa. Number in thousands per c.mm. :				
(*a*) mean	460 (1)	844 (6)	936 (24)	—
(*b*) range		320-1875	431-2010	
Total number in millions per ejaculate :				
(*a*) mean		6313	4970	
(*b*) range		1700-15000	1160-11055	
Percentage abnormal spermatozoa :				
a) mean	2·0 (1)	9·9 (8)	11·1 (11)	
(*b*) range		6-14	5-18	

Figures in parentheses indicate number of ejaculates.

from these rather insignificant differences, it seems more important to regard this group of eight bulls as of fertility ranging from 1·18 to 2·71 services per conception with a mean of 1·79, and to examine the ejaculates more particularly for the range of variation that occurs in the different criteria.

Data on the volume of the ejaculates of the different bulls are given in Table II. The mean volume of the ejaculates for the two other bulls was : bull Sl. 5·3 mls. (71 ejaculates) and bull A3 5·5 mls. (11 ejaculates). The range of variation for all ejaculates from the eight bulls was 1·5 mls. to 12·0 mls.

The range of variation in motility is shown in Table IV. Thirty-five ejaculates (7·2 per cent.) had a motility of less than 70 per cent. When the first ejaculate was of poor motility (less than 70 per cent.), the second ejaculate was usually better, but in several instances the second ejaculate showed no improvement over the first. It has been observed lately that a bull may ejaculate two specimens of poor motility and yet the third specimen may be of good motility. It is therefore necessary to recognise that a certain number of ejaculates from fertile bulls may be of poor motility, but that when motility is poor on the first ejaculation it tends to improve on repeated ejaculation.

Similarly with the concentration of spermatozoa. Out of 484 ejaculates, two ejaculates (one from bull H and one from bull A2) contained no spermatozoa. The specimen from bull H was a single ejaculate, that from bull A2 was the first ejaculate and the second was a good dense specimen of high motility.

In addition to this general analysis a closer examination of the relationship between the quality of the semen and fertility can be made by examining the ejaculates actually used for insemination. No ejaculates were used for insemination which showed a motility of less than 70 per cent. There was no difference in fertility when the motility varied from 70 to 100 per cent. (Table X *over-leaf*). Donham, Simms and Shaw (1931), who collected sperm from the vagina of the cow found a correlation between motility and fertility.

It was thought at one time that the keeping quality of sperm might give an indication of its fertilising capacity (Anderson, 1939), but it now appears that this is not so (Table XI (*over-leaf*)). No correlation was

Table X

Motility and Fertility

Per cent. Motility	Inseminations Number	Per cent. Fertile
10	212	49·1
9	131	59·5
8	119	54·6
7	23	43·5
Mean 93	485	53·0

number, regarded arbitrarily as of reduced fertility, because their sperm was below normal in one or other respect, ejaculates of motility of 70 per cent. and over had an average of 18·8 per cent. of abnormal spermatozoa (range 3 to 31); ejaculates of motility of less than 70 per cent. had an average of 11·2 per cent. of abnormal spermatozoa (range 4 to 30). Three of the seven bulls whose ejaculates were of good motility (70 per cent. and over) had 20 per cent. or more abnormal spermatozoa and one of the eight bulls whose sperm was of poor motility (below 70 per cent.) had 30 per cent. abnormal spermatozoa. Thus, bulls whose ejaculates are of good motility may contain a high

Table XI

Fertility of Fresh Sperm and Motility of Stored Sperm

Inseminations No.	Per cent. Fertile	Per cent. Motility at 24 Hours	Inseminations No.	Per cent. Fertile	Per cent. Motility at 48 Hours	Inseminations No.	Per cent. Fertile	Per cent. Motility at 72 Hours
16	43·8	90 to 100	2	0	90 to 100	3	66·6	90 to 100
52	50·0	70 to 80	18	38·9	70 to 80	3	66·6	70 to 80
7	71·4	50 to 60	39	56·4	50 to 60	24	41·7	50 to 60
3	33·3	30 to 40	9	66·6	30 to 40	17	47·1	30 to 40
—	—	—	1	0	10 to 20	3	66·6	10 to 20
—	—	—	—	—	—	20	60·0	N

found between the fertility from insemination with fresh sperm and the motility of this sperm after storage for 24 to 96 hours. The spermatozoa counts made on ejaculates used for insemination are too few for an examination of the relation between sperm density and fertility.

The general conclusion is that none of the criteria used was adequate to determine small differences in grades of fertility. Edwards and Walton (1939) came to a similar conclusion. On the other hand, a type of sperm with certain general features, which can readily be recognised, is associated with fertility. A corollary is that semen from certain bulls may show a wide variation in certain characteristics. Such cases, however, appear to be relatively few in number and their significance can be better judged if a number of ejaculates are examined.

General.—It is of interest to examine, carefully, those cases in which sperm is judged as good, by certain criteria and as poor, by others. In the first place are those bulls whose sperm may be judged as perfectly normal by all available criteria, yet whose fertility is low and there may be a high incidence of abortions following their services. Williams, W. W. (1920) mentions several such cases. Lagerlöf (1934) described a sterile bull (No. 86) whose ejaculates were perfectly normal in respect of number of spermatozoa, motility and percentages of abnormal and immature spermatozoa, but they contained streptococci. Such cases are probably exceptional. They do not invalidate the available criteria for evaluation of sperm, but because of them it is necessary to recognise that fertility cannot be guaranteed from sperm examination.

In the previous series of sperm examinations (Anderson, 1939, 1940) it was found that the ejaculates of eleven bulls with epididymitis, which showed either no motility or motility of 10 to 20 per cent., contained a percentage of abnormal spermatozoa ranging from 4 to 66. In these cases, motility was a better guide than morphology. In another class of bull, 15 in percentage of abnormal spermatozoa and bulls whose ejaculates are of low motility may contain only a small percentage of abnormal spermatozoa.

Lagerlöf (1934) has published detailed accounts of semen examinations from bulls whose breeding history at the time of the examination was known. A critical examination of his data permits an interesting comparison on the value of the different indices. Thus, in nine sterile bulls with hypoplasia of the testes, spermatozoa were few or absent, the percentage of abnormal and immature spermatozoa was high; the motility was less than normal, except in one bull in which it was classed as "good." This bull had an average of 115,000 spermatozoa per c.mm. Thus at least eight of these nine bulls could have been classified on sperm density and motility as well as on morphology of the spermatozoa. In bulls with degenerative changes in the testes, however, there were two bulls (Nos. 15 and 36) in which the density and motility were normal, but the percentage of abnormal spermatozoa was high. One of these bulls was of poor fertility and the other was sterile. Another bull (No. 56), similarly affected, had a normal density of spermatozoa, but poor motility and a high percentage of abnormal spermatozoa. One bull (No. 8) with *abortus* orchitis had almost a normal concentration of spermatozoa (650,000 per c.mm.), few abnormal types of spermatozoa, but the motility was almost nil. Generally, there was a correlation between the density of the spermatozoa, the percentage of abnormal spermatozoa and the motility, but this association did not hold in every case, as the above examples show.

Practical Consideration

When widespread sterility occurs in bulls, as it does in Kenya, a demand arises to have the fertility of bulls tested, either in connection with suspected sterility on a particular farm or in connection with the purchase of bulls. The question naturally arises, to what extent is it possible to-day to determine with any accuracy the fertility of a male animal?

Fertility in the bull may be estimated from (1) previous breeding records, (2) clinical examination of the genital organs, and (3) examination of the semen. Breeding records refer to the past history of an animal and are therefore of value only for that purpose. When based on calvings they refer to a period ante-dated by at least nine months. A bull sterile to-day, may have been of normal fertility nine months ago. A closer estimate may be attempted by basing fertility on cows " holding," but even if this method were sufficiently accurate, there is still an intervening period during which fertility may have become affected. Bull S provides an example of the rapidity with which a bull may become sterile.

Clinical examination of the genital organs is a useful adjunct only. It is possible to detect changes in the size, shape and consistency of the testes, epididymes and in some cases in the seminal vesicles, and such abnormalities are probably associated with alteration in fertility, but sterile bulls and bulls of low fertility may have, as far as can be detected clinically, perfectly normal genital organs. It is therefore on the examination of the semen that judgment must be based. Much valuable work has been done on this subject in recent years and it is possible to identify a characteristic type of semen with fertility, sterility or with " reduced " or " doubtful " fertility. Milovanov (1936) has summarised the views of workers on the evaluation of the sperm of fertile sires and the author's results are very similar. The extreme cases of sperm evaluation are those, (*a*) in which the ejaculate is useless for impregnation, since it contains either no spermatozoa or only dead spermatozoa and (*b*) in which the ejaculate, examined by all the available criteria, is " good " and conforms with that of fertile bulls. In the latter class of ejaculate it is not yet possible to identify a particular type of ejaculate, nor an ejaculate showing certain features, with a particular degree of fertility. None of the criteria commonly used for evaluation of sperm is adequate for this purpose. The most promising approach to this problem is the work of Edwards and Walton (1939) who have found a relationship between the " respiration rate " of sperm and the number of services required for conception.

With the intermediate types of sperm the position is by no means clear. A particular ejaculate may fail in respect of one or more criteria and is therefore regarded as below the standard normally associated with fertility. When the concentration of spermatozoa is low, the motility very poor and the percentage of abnormal spermatozoa high it is likely that the fertility is low or even absent. It does not, however, appear possible at present to draw a hard and fast line between fertility and sterility on the basis of sperm examinations.

A point to be decided is whether or not a particular ejaculate can be regarded as typical of a bull. This is of particular importance when a bull is unwilling to ejaculate more than once when tested. When a single ejaculate, judged by available criteria, is classed as " good," the bull can probably be regarded as fertile, but a poor ejaculate does not by any means condemn the bull. Several instances have been noted in which a very poor ejaculate has been obtained from fertile bulls. In such cases, if possible, two or more ejaculates should be obtained. If the sperm is now good, the bull can probably be regarded as fertile, but if there is no improvement, or the sperm is worse, the bull is regarded as doubtful. In such cases repeated tests at, say, monthly intervals should be made to determine if the bull is improving or deteriorating. Some bulls may temporarily produce poor or bad sperm but in time return to normal ejaculates. Lagerlöf (1934) mentions two bulls which required two to three months for the sperm to return to normal. The extent to which this is possible will depend on the degree of involvement of the testes and accessory reproductive organs. When the ejaculates are poor a clinical examination of the genital organs may aid diagnosis and prognosis.

Summary

An account of the examination of the semen of clinically normal and abnormal bulls in Kenya is given. The sperm characteristics of bulls whose breeding history was known at the time of examination are discussed.

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FURTHER INVESTIGATIONS ON ARTIFICIAL INSEMINATION OF CATTLE

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FURTHER INVESTIGATIONS ON ARTIFICIAL INSEMINATION OF CATTLE

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A PRELIMINARY investigation of artificial insemination of cattle in Kenya was made during 1936 and part of 1937 (Anderson, 1938). In this paper experiments up to August 1939 are described. The earlier results are repeated here for comparison and also because the results from farms 1 and 2 in the previous report were based on cows 'holding' and not on actual calvings.

MATERIAL AND METHODS

The technique used, which is similar to that of Walton (1936) and Russian workers, has already been fully described (Anderson, 1937, 1938). The bulls which provided the sperm were mostly pure-bred bulls and the cows were all high-grade cows, except for one experiment with Zebu cows. In the Experimental Station herd the type was predominantly Shorthorn; on farm 1, Friesian; and on farm 2, Ayrshire. All the results in the paper are based on calvings.

RESULTS

The results of the experimental investigation of artificial insemination on three farms are shown in Table 1.

Table 1

Farm	No. of cows	Calved		No. of insemina-tions	No. of inseminations	
		No.	%		Per conception	Per cow
E.S.	451	385	85·4	662	1·72	1·47
1	185	108	58·4	273	2·53	1·48
2	97	70	73·7	123	1·76	1·29
	Total 733	563	76·8	1058	1·88	1·44

The herds on farms 1 and 2, particularly that on farm 1, were affected by the contagious venereal disease peculiar to Kenya, which is associated with vaginitis in the cow and epididymitis in the bull (Daubney *et al.* 1938; Anderson, 1939, 1940). It is believed that the prevalence of this

disease may have been responsible for the low percentage of calvings on farm 1. Artificial insemination has been practised on this farm since the end of the above experiment in March 1937, by the Manager, and the calvings are said to be satisfactory. On farm 2, subsequent to the above experimental period, a further twelve cows calved to artificial insemination giving a calving percentage of 86·3.

The Experimental Station herd has never been affected by venereal disease, and since 1935 it has been free from contagious abortion. The annual percentage of cows calving to artificial insemination in this herd has been satisfactory (Table 2). 80 % fertility was obtained in this herd in the period 1929–36 from ordinary service.

Table 2

Year	No. of cows	Calved	
		No.	%
1936	83	69	83·1
1937	114	104	91·5
1938	133	117	88·0
1939	121	95	78·6

The insemination records of sixteen bulls are shown in Table 3. The number of inseminations required for conception was similar on the Experimental Station and farm 2; on farm 1 the number required was higher because of genital disease in the cows.

Table 3

Farm	Bull	Breed	No. of inseminations	No. of conceptions	No. of inseminations per conception
E.S.	S 1	Shorthorn grade	173	96	1·80
	Ag	Ayrshire grade	40	18	2·22
	A 2	Ayrshire	42	19	2·21
	A 3	Ayrshire	19	7	2·71
	H	Hereford	92	61	1·51
	AA	Aberdeen-Angus	71	45	1·58
	Su	Sussex	45	18	2·50
	F	Friesian	20	17	1·18
			Total 502	281	1·79
1	A	Friesian	94	43	2·19
	B	Friesian	104	43	2·42
	C	Friesian	75	22	3·41
			Total 273	108	2·53
2	A	Ayrshire	28	19	1·32
	B	Ayrshire	30	20	1·33
	C	Ayrshire	40	23	1·74
	D	Ayrshire	17	6	2·83
	E	Ayrshire	8	2	4·00
			Total 123	70	1·76

Table 4

	E.S.		Farm 1		Farm 2	
	No.	%	No.	%	No.	%
Cows calved to: 1st insemination	241	67·1	95	73·1	54·	77·2
2nd insemination	76	21·2	24	18·4	14	20·0
3rd insemination	23	6·4	9	7·0	2	2·8
4th insemination	11	3·1	2	1·5	—	—
5th-7th insemination	8	2·2	—	—	—	—

From 2 September 1937 to 1 December 1937, 118 Zebu cows were artificially inseminated, and 174 inseminations were performed, i.e. 1·47 per cow using Experimental Station bulls. Seventy cows calved (59·3 %), giving a ratio of 2·43 inseminations per conception.

On farm 1 there was no significant difference in the number of inseminations required for conception, using a dose of 1 ml. undiluted sperm, 1 ml. sperm diluted $\times 2$, and 1 ml. sperm diluted $\times 4$. An average of 2·5 inseminations per conception were required on this farm which is high compared with the two other herds. On farm 2 there was also no significant difference in the number of inseminations required for conception. In the Experimental Station herd, on the other hand, there were highly significant differences between the different experiments ($\chi^2 = 19{\cdot}6$ for 3 degrees of freedom, which exceeds the 1 % point). The number of inseminations required for conception varied from 1·30 with

Table 5

Procedure	Farm	No. of cows	No. of insemina-tions	Calved		No. of inseminations	
				No.	%	Per conception	Per cow
			Single insemination				
1 ml. undil.	E.S.	181	260	127	70·2	2·05	1·44
	1	28	42	17	60·7	2·24	1·50
	2	76	91	52	68·4	1·75	1·20
	Total	285	393	196	68·8	2·01	1·38
0·5 ml. undil.	E.S.	67	73	56	83·4	1·30	1·09
0·5 ml. dil. $\times 2$	E.S.	70	74	42	60·0	1·76	1·06
1·0 ml. dil. $\times 2$	E.S.	68	91	52	76·5	1·75	1·49
	1	87	103	40	46·0	2·58	1·17
	2	30	32	18	60·0	1·78	1·07
	Total	185	226	110	59·5	2·05	1·22
1·0 ml. dil. $\times 4$	1	97	128	51	52·5	2·51	1·32
			Double insemination				
0·5 ml. undil.	E.S.	56	56	38	67·9		
1·0 ml. undil.	E.S.	79	100	62	78·5		

a dose of 0·5 ml. undiluted sperm to 2·05 with a dose of 1·0 ml. sperm also undiluted.

But, although these differences were significant in the Experimental Station herd for the period 1936–9 they were not confirmed, when with the object of eliminating any possible seasonal effects on fertility, the experiments were repeated during September 1938 to March 1939 (Table 6).

Table 6

Procedure	Period	No. of cows	No. of inseminations	Calved No.	Calved %	No. of inseminations per cow
1 ml. undil.	19. xi. 38–9. iii. 39	42	43	29	69·0	1·48
0·5 ml. undil.	5. ix. 38–27. i. 39	27	31	19	70·4	1·43
0·5 ml. ×2 undil.	30. i. 39–17. iii. 39	21	22	11	57·1	2·00

$\chi^2 = 1{\cdot}88$.

Two inseminations in the one heat period did not give better results than a single insemination. 69 % of forty-two cows which received 1 ml. of undiluted sperm calved (Table 6), compared with 68 % of fifty-six cows which received two inseminations of 0·5 ml. sperm in the one heat period (Table 5).

DISCUSSION

It is believed that when highly fertile bulls are used with normal, healthy cows about 1·5–2·0 services are required per conception (Anderson, 1938, 1939). The figures obtained from artificial insemination in different countries mostly fall within this range. Davis (1939), for example, found a variation of from one to four inseminations per conception for different bulls with an average of 1·69, and Henderson's (1939) figures for individual bulls varied from 1·39 to 2·69 inseminations per conception with an average of 1·91. There was considerable variation in the number of inseminations required for conception in the Experimental Station herd and on farms 1 and 2, but the mean figures for the Experimental Station and farm 2 were within the normal range. On farm 2 the figure was raised by genital infection.

On the basis of 1·5–2·0 services per conception, from 50 to 67 % of cows should conceive to a single insemination. In Sorenson's (1938) 1936–7 experiment, of 1157 cows, 555 conceived to the first insemination, i.e. 49 %, a figure similar to that obtained by Bell (1938) whose cows were inseminated once only and 51·3 % conceived; 67 % had conceived by the second insemination, 75·9 % by the third and 78·8 % by the fourth. Bonadonna (1939) stated that pregnancy was obtained in

60–80 % of cases with a single insemination, and up to 90 % with one, two or three inseminations. In the Experimental Station herd about 58 % of the inseminations were fertile.

Of cows that conceived, Sorenson found that 59·2 % did so at the first insemination. Davis found that 66·7 % resulted from one insemination, 21·5 % from two inseminations, 7·5 % from three, 8·9 % from four and 1·9 % from five inseminations. Very similar results were obtained in the Experimental Station herd (Table 4).

To obviate different levels of fertility in the three herds it is necessary to compare experiments in each herd. On farms 1 and 2 there was no significant difference between the use of undiluted sperm and sperm diluted up to a maximum of $\times 4$ with dilutor GTC. In the Experimental Station herd there was a significant difference between experiments using different doses and dilutions of sperm over the whole period reported in this paper. These experiments, however, were carried out under different conditions, at different times of the year, using sperm from different bulls. The results are not therefore solely attributable to dilution and dose of sperm. In fact, when the experiments were carried out at much the same period of the year no significant differences were found between using 1 ml. of undiluted sperm, 0·5 ml. of undiluted sperm, and 0·5 ml. of sperm diluted $\times 2$.

Russian workers (Andreev, 1937; Kirillov, 1937) found that two inseminations during a single heat period gave better results than a single insemination. This observation does not apply to grade cows in Kenya, perhaps because of the relatively short period of heat experienced by these cows.

Summary

An account is given of artificial insemination of 733 grade cows in Kenya from January 1936 to August 1939. 76·8 % of the cows inseminated calved, with an average of 1·88 inseminations per conception. The different doses and dilution of sperm used gave very similar results, as did two inseminations, compared with one insemination, in a single heat period.

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FURTHER INVESTIGATIONS ON ARTIFICIAL INSEMINATION OF SHEEP

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FURTHER INVESTIGATIONS ON ARTIFICIAL INSEMINATION OF SHEEP

By J. ANDERSON

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Preliminary experiments on artificial insemination of high-grade Merino sheep in Kenya were made in 1935–6 (Anderson, 1937). Further experiments made from 1936 to 1939 are reported in this paper.

As far as the author is aware, very little work has been done on artificial insemination of Merino sheep. A small number of ewes have been artificially inseminated in Australia (Gunn, 1936; Kelley, 1937) and in South Africa (Quinlan *et al.*, 1936). Gunn inseminated 136 Merino ewes and thirteen lambed. The procedure adopted, however, was not such as would give the best results, e.g. in some cases the sperm was collected from the vagina of a ewe; physiological saline solution was used for diluting sperm; and in some cases, sperm was kept for as long as 6 hr. at room temperature before use. Kelley (1937) obtained 22 % of lambs from artificial insemination of fifty-five ewes. In the first experiment made by Quinlan *et al.* in South Africa, sperm was collected from the vagina of a ewe and 1–2 ml. sperm was diluted with 7–8 ml. Tyrode solution, 1 ml. of which was introduced into the vagina. Of twenty-five ewes inseminated with fresh diluted sperm fourteen (56 %) lambed. In the second experiment an artificial vagina was used for collecting the sperm. The sperm was diluted 1 in 3 with GPS-8, and inseminations were carried out within $\frac{1}{2}$ hr. of collecting the sperm. A single insemination was made into the cervix at one heat period. Of forty ewes inseminated nineteen (47·5 %) lambed. There was a difference of 10 % in favour of hand-service. These workers suggest that driving of sheep, which causes a rise in temperature, before insemination, may have reduced the percentage of pregnancies. These results, however, are very similar to those which have been obtained at Naivasha, and it is probable that the low percentage of conceptions is related, partly at least, to the low fertility of Merino sheep.

Quinlan and co-workers state that the technique of artificial insemination is so complicated that it is unlikely to be of economic value to the rural population of South Africa in the normal breeding of domestic

animals, but more intensive use may be made of exceptional sires in large stud flocks and the services of an experienced technician will be warranted. The author does not agree with this view as far as Kenya is concerned. The technique of artificial insemination is not so complicated that it cannot easily be put into practice by the average person after a short period of training. The results obtained experimentally in Kenya have shown that artificial insemination can be successfully applied to sheep in Kenya, and good results have also been obtained by those farmers who have themselves used the method.

MATERIAL AND METHODS

The rams used were all pure-bred Merinos and the ewes were high-grade Merinos, which have been bred up in Kenya for many generations by crossing a local fat-tailed type of ewe with imported Merino rams.

Vasectomized rams were used to pick out ewes on heat. Details about the time of insemination during oestrus, the number of inseminations during each oestrous period, the degree of dilution of sperm, and the dose of sperm are given in the appropriate places, later in the paper. The technique used, which is similar to that of Walton (1936) and Russian workers has already been described (Anderson, 1937, 1938).

RESULTS

The total results obtained from artificial insemination of sheep at the Experimental Station, Naivasha, for the period 1935–9 are shown in Table 1.

Table 1

Period	Farm	No. of ewes	Ewes lambed	
			No.	%
1935–9	E.S.	1817	1183	65·1
1936, 1937	W	4203	2106	50·1

The results from 'E.S.' (Experimental Station) ewes were better than from the 'W' ewes, but they are both below what is normally obtained from ordinary service. Results comparable with those from ordinary service have however, been obtained from certain experiments, and it is now known that the procedure adopted in some of the experiments was not such as would give the best results, as is explained later (Table 2).

No attempt was made to inseminate the maximum number of ewes with one ram. Nevertheless, the number of rams actually used was

Table 2

	1935–6	1936	1937	1938	1939	1935–9
Av. no. of ewes inseminated by 1 ram	202	652	456	131	300	348
Av. no. of lambs born to 1 ram	150	201	297	61	245	191

considerably less than formerly, when ordinary service was practised. Previous to 1935, eighteen rams were kept for a flock of about 600 ewes, and of these rams eleven were pure-bred Merinos and seven were grade Merinos. From 1935 to 1939 an average of three rams was used per breeding season. The number of ewes inseminated on an average by one ram and the number of lambs born on an average to one ram as the result of artificial insemination thus showed a considerable increase over that obtained from ordinary service. In the period 1935–9 an average of 148 ewes were inseminated by one ram and an average of 191 lambs were born to one ram. In 1937 one ram sired 411 lambs.

Season. The results obtained with the Experimental Station ewes at different periods of the year are given in Table 3. The better results

Table 3

			Experimental ewes	
Period of insemination	No. of ewes inseminated	% of ewes lambed	Length of oestrous cycle in days	% of ewes on heat
April-June, 1937	462	79	19·3	100
April-July, 1939	601	75	17·3	92
Dec. 1935-Feb. 1936	202	74	18·6	—
June-Aug. 1938	393	47	24·6	87
June-Aug. 1936	159	25	29·2	66

compare favourably with the normal lambing from such sheep after ordinary service. On two occasions, however, the results have been unsatisfactory. It is of interest to compare the results from artificial insemination with the length of the oestrous cycle and the incidence of oestrus in experimental ewes (author, unpublished results) for the same periods of the year (Table 3). There is a very distinct correlation between the percentage of ewes that lambed and the length of the oestrous cycle and the percentage of ewes on heat. The longer the cycle and the smaller the percentage of ewes on heat, the poorer was the lambing. Better results were obtained from artificial insemination at those periods of the year when the oestrous cycle was short and the incidence of oestrus was high. The main factor in obtaining a satisfactory lambing was therefore the season of the year at which artificial insemination was practised.

It was observed in 1938 that considerable variation occurred in the incidence of lambing. 166 ewes were inseminated twice in the one oestrous period from 12 July to 21 August, omitting the period from 9 to 15 August (Table 4). 69·3 % of these 166 ewes came on heat in

Table 4

Period	No. of ewes	% of total ewes on heat	% of ewes lambed
12–18 July	31	18·7	12·9
19–25 July	58	34·9	36·2
26 July–1 Aug.	26	15·7	42·3
2–7 Aug.	37	22·3	16·2
17–21 Aug.	14	8·4	50·0

20 days and 91·4 % in 27 days. It seems that conditions were especially favourable for conception during certain short periods in the course of this experiment.

Dilution of sperm. Sperm used undiluted, and diluted up to a maximum of ×8 has proved equally effective; beyond this point there was a marked reduction in the percentage of conceptions (Table 5). All experiments using a dilution of ×8 have not, however, given similar results; the percentage of conceptions in different experiments varied from 20 to 54.

Table 5

	Dose 0·2 ml. sperm undiluted	Sperm diluted				Dose 0·1 ml.
		×3	×4	×8	×16	×8
No. of ewes	47	50	65	925	669	533
No. of ewes lambed	22	21	29	412	115	81
% of ewes lambed	46·8	42·0	44·6	44·5	17·2	15·1

Cooling of sperm. The cooling of sperm had little effect on the conception rate (Table 6).

Table 6

	No. of ewes	% lambed
Sperm cooled to 10° C. and used cool	47	17·0
Sperm cooled to 10° C. and warmed by hand before use	62	17·8
Sperm used uncooled	57	22·0

Number of inseminations. Up to 1937 season the ewes were inseminated once during each heat period and thereafter twice or more (twice unless otherwise stated). The comparison between 1937 and 1939 is shown in Table 7.

In 1937 the ewes were picked out in the morning and inseminated once only in the morning. In 1939 the ewes were picked out in the

Table 7

No. of oestrous periods ...	1st	2nd	3rd	4th	5th
	April-June, 1937				
No. of ewes	1516	1171	782	483	136
% of ewes lambed	19·5	21·3	23·5	22·6	27·9
	June-August, 1939				
No. of ewes	601	408	288	81	—
% of ewes lambed	27·6	29·3	32·6	25·9	—

morning, and during the day up to about 3 p.m. and these ewes were inseminated twice, once that evening and again the following morning. The results from this latter procedure were better than from the former.

Table 8

	June-August, 1938	
	No. of ewes	% lambed
Single insemination (*a*)	27	14·8
Single insemination (*b*)	29	3·5
Single insemination (*c*)	29	6·9
Double insemination (*d*)	166	29·5

(*a*) Ewes picked out a.m. and inseminated that p.m.
(*b*) Ewes picked out a.m. and inseminated the following a.m.
(*c*) Ewes picked out a.m. and inseminated the following p.m.
(*d*) Ewes picked out a.m. and inseminated that p.m. and following a.m.

Results very similar to those obtained in 1939 were obtained from a double insemination in 1938. Single inseminations at different times in relation to oestrus gave poor results (Table 8).

Table 9. *One or more inseminations in the one heat period*

No. of inseminations	No. of ewes	% lambed
1	5	0
2	56	37·5
3	16	37·5
4	19	48·4
Total	96	37·5

In Table 9 is shown the results from inseminating ewes, once, twice, three times and four times, according to the length of oestrus. The ewes were picked out in the morning and inseminated; if still on heat that evening they were again inseminated; if still on heat the following morning they were inseminated a third time and so on. This experiment, which lasted for 14 days from 25 November to 8 December 1937, gave a 37·5 % lambing. One ram was used for all the ewes. However, on occasion a single insemination has given equally as good or better results

as two or more inseminations in the one heat period, as for example in 1935–6 (Anderson, 1937), and in the experiments detailed above using sperm undiluted and diluted up to ×8. It is probable that this fact is related to the period of the year at which the inseminations were carried out.

Ordinary service in August 1936 of 309 ewes (the ewes were run with rams for 3 weeks) gave a 74% lambing and of 96 ewes (hand service) gave 70% lambing (author, unpublished results). There was thus little difference between a single service and several services, but the results from a single service are better on the average than the results from a single insemination. Artificial insemination in June-August 1936, using a single insemination in each heat period, gave poor results (25% lambing) compared with a single service per heat period (70% lambing).

The percentage of fertile inseminations for different rams in 1936 and 1937 is shown below. The results are not strictly comparable since the inseminations were not all made at the same period and different dilutions, some of which were too high, were used.

Table 10

	1936			1937	
Rams	57	708	3237	3062	3068
No. of inseminations	344	77	67	1993	732
No. of inseminations fertile	150	8	13	453	139
% of inseminations fertile	43·6	10·4	19·3	22·7	19·0

Time of insemination in relation to oestrus. Two experiments, involving 242 ewes, were carried out in 1938 and 1939, to determine the conception rate when ewes were inseminated at different intervals from the beginning and end of oestrus. The results are shown in Table 11.

The actual conception rates in the two experiments are not strictly comparable since the ewes were inseminated at different periods of the year in 1938 and 1939 and different rams were used. The experiments show that the period from 12 to 30 hr. after the onset of oestrus was the most favourable to conception. During this period in 1938, 25% of the ewes conceived, and in 1939, 24% conceived.

The duration of oestrus in the inseminated ewes in 1938 was 20·8 hr. The duration of oestrus is not known for the inseminated ewes in 1939, but for other experimental oestrous cycle ewes at the same period of the year it was 28·6 hr. There is a minimum period of about 26 hr. before which ovulation does not occur in grade Merino ewes in Kenya (Anderson, 1938*b*), and when oestrus lasts longer than about 26 hr. ovulation occurs

Table 11

Period of oestrus at which ewes inseminated hr. from onset of heat	1938		1939	
	No. of ewes	% lambed	No. of ewes	% lambed
1–6	20	25·0	25	0
12–18	28	42·9	25	20
24–30	32	9·4	25	24
36–42	29	0	25	12
48–54	32	0	—	—

No. of ewes	No. lambed	Duration of oestrus in hr. Mean	Standard deviation	Range
Insemination 1–13 hr. after the end of oestrus				
27	12	14·8 ±0·67	3·5	10·0–22·0
18	1	18·2 ±1·01	4·4	8·2–26·6
9	0	31·5	—	29·6–36·0
Insemination 14–39 hr. after the end of oestrus				
50	0	17·9 ±0·29	6·09	6·8–30·1

very shortly after the end of oestrus. The longer period of oestrus in the 1939 experiment therefore probably explains the somewhat later interval after the onset of oestrus at which most conceptions occurred.

Insemination after the end of oestrus is effective when the length of oestrus is short, and within the limits of the experiment, the shorter the average duration of oestrus, the higher was the conception rate, when insemination was performed 1–13 hr. after the end of oestrus. No conceptions occurred when insemination was carried out 14–39 hr. after the end of oestrus, which lasted on the average for 18 hr.

For the thirteen ewes which lambed from insemination 1–13 hr. after the end of oestrus, the mean duration of oestrus was 14·7 hr.; the interval between the end of oestrus and insemination was 8·3 hr.; and the interval between the onset of oestrus and insemination was 23 hr. These results must be considered in relation to the time of ovulation. In the thirteen ewes with short oestrous periods, which were inseminated 1–13 hr. after the end of oestrus, the sperm was introduced a few hours before the probable time of ovulation. In the nine ewes whose mean length of oestrus was 31·5 hr. and which were inseminated 1–13 hr. after the end of oestrus, and in the fifty ewes whose mean length of oestrus was 17·9 hr. and which were inseminated 14–39 hr., the sperm was introduced after the probable time of ovulation.

Thirty-five ewes were inseminated 8–23 hr. before the end of oestrus and six lambed. The mean length of oestrus in the six ewes that lambed was 23·2 hr. and the mean interval between insemination and the end

of oestrus was 14·7 hr. Of nineteen ewes inseminated up to 19 hr. before the end of oestrus two lambed; of eight ewes inseminated 13–18 hr. before the end of oestrus two lambed; and of eight ewes inseminated 19–22·5 hr. before the end of oestrus two lambed. Ram sperm thus remained alive and fertile in the genital tract of the ewe for 22·5 hr., the approximate time between insemination and ovulation.

DISCUSSION

Season. It is well known that Merinos in certain parts of Australia and South Africa, when conditions are favourable, experience a continuous series of dioestrous cycles throughout the year (Marshall, 1922; Quinlan & Mare, 1931). According to Quinlan & Mare (1931) this occurs under Great Karroo conditions in South Africa. Kupfer (1929), on the other hand, observed the existence, under western Free State conditions in South Africa, of a prolonged anoestrous period in Merino ewes. Roux's (1936) observations agree to a very great extent with those of Kupfer. In Australia, Kelley & Shaw (1939) have shown that Merino ewes have a well-defined periodicity in the percentage of ewes coming on heat. There was a fall in the incidence of oestrus in the spring months, followed by a rise in the summer months, the higher levels being maintained during late summer and autumn (Australian seasons).

In Kenya, there is a marked seasonal variation in reproductive capacity in Merino ewes, as illustrated by the length of the oestrous cycle and the incidence of oestrus (author, unpublished results). These seasons are not sharply defined in different years, nor are they of the same extent, nor do they occur at exactly the same period in each year. Nevertheless this seasonal variation must, as far as possible, be taken into account in breeding operations. Some years and certain seasons of the year are more favourable for full expression of sexual activity in Merino ewes. In general, it may be said that it is in the early months of the year that the incidence of oestrus is highest and the cycle shortest in grade Merino ewes in Kenya.

It is therefore at this period of the year that the best results should be obtained from breeding operations. The results from artificial insemination in different seasons of the year support this view (Table 3). In a good year, however, the season favourable to reproduction may be greatly extended.

It is the normal practice in the Rift Valley district in Kenya to put the ewes to the ram in May and June. This season has been determined

solely by the welfare of the lambs, in that they do better and are less subject to helminth infection when born towards the end of the year. With better methods for control of worm infestation it may eventually prove advisable to alter the breeding season to a period somewhat earlier in the year.

One of the biggest difficulties in artificial insemination of sheep in Kenya has been the failure of ewes that did not conceive to return to the ram after the normal interval of about 17–19 days. If ewes which fail to hold to insemination (or service) do not return to the ram at normal intervals, they will have less chances for re-insemination during the breeding season and a poorer lambing will consequently result. In June-August 1936, only 25 % of 159 ewes lambed, yet 75 % of these ewes did not return to the ram. In the Experimental Station flock abortion is rare, but it cannot at present be decided whether or not conception and subsequent interruption of pregnancy may account for some of the cases of failure to return to the ram. The most probable explanation available at present, however, for failure of inseminated ewes to return to the ram, is the great seasonal variation that has been observed in the length of the oestrous cycle.

The percentage of conceptions to insemination during one heat period has never been greater than about 50 %. In April to July 1939, for example, when each ewe was inseminated twice during the one heat period the average percentage of conceptions was 29 %. To obtain the best results from artificial insemination in high-grade Merino ewes in Kenya, it is therefore essential to get as many ewes in lamb as possible when they first come on heat, and highly important, to ensure as far as possible, that those ewes which do not conceive will return to the ram after approximately the normal cyclical interval, for re-insemination. The main factor in determining this latter point is a seasonal one.

Even during a restricted breeding season changes occur in the reproductive activity of sheep. Grant's (1934) data show a distinct trend to longer cycles as the breeding season advances, and according to Chapman & Casida (1937), who examined these data statistically, this increase from month to month accounts for 37 % of the variation in the oestrous cycle length in this group of sheep. McKenzie & Terrill's (1937) data also show that the length of the oestrous cycle increases during the breeding season. McKenzie & Terrill noted a general tendency for shorter oestrous periods to occur near the beginning and end of the breeding season. There is a tendency for shorter oestrous periods to occur near the beginning and end of the breeding season and the occurrence of ovulation

without oestrus has been noted preceding and following the breeding season (Grant, 1934; Cole & Miller, 1935; McKenzie & Terrill, 1937).

There is also evidence that the fertility of sheep changes during the breeding season. It has been observed that twins are usually born early in the breeding season (Heape, 1899; Marshall, 1905). In American flocks of Shropshire sheep, Roberts (1921) found that the percentage of multiple births was higher in the earlier part of the lambing season, and in British flocks, Nichols (1924) noted that by far the greater number of multiple births occur at a time corresponding to that at which the ewes go fastest to the ram, and although this time varies a certain amount according to the treatment the ewes and rams have received before mating, it is usually at the beginning of the breeding season. It thus appears, as Marshall states, that the reproductive activity of the ewe tends to be greatest at the beginning of the breeding season. It is not therefore surprising that, if variation in fertility occurs during a relatively short restricted breeding season, it should also occur in sheep, such as grade Merinos in Kenya, which can breed throughout the year.

Time of insemination. The optimum time for insemination depends mainly on (1) the vitality of spermatozoa in the genital tract of the ewe, and (2) on the vitality of the ovum. The consensus of opinion is that the life of the ovum is very short. Hartman (1932), in reviewing the evidence, states that 'facts are accumulating which show that the time of survival of the unfertilized ovum is measured in hours, not days.' The data of Quinlan *et al.* (1932) indicate that the ovum rapidly loses its vitality and is available for fertilization for a few hours only after ovulation. Kuznecov (1934) holds a similar view.

In grade Merino ewes in Kenya, ovulation occurs shortly after the end of oestrus, but there is a minimum period of about 23–25 hr. after the onset of oestrus before which it does not occur, even if the duration of oestrus is much less than this interval. Anderson (1938*b*) and McKenzie & Terrill (1937) found that, although there is some variation in the time of ovulation, generally speaking, it takes place near the end of oestrus. In Merino ewes Kelley (1937) found that ovulation took place after the end of oestrus.

The experiments reported in this paper on insemination after the end of oestrus, support the view that the life of the ovum is short. In the nine ewes whose mean oestrous period was 31·5 hr., ovulation presumably occurred about the end of oestrus. They were inseminated 1–13 hr. after the end of oestrus and after the probable time of ovulation, and none conceived. In the fifty ewes, whose mean oestrous period was

17·9 hr., ovulation probably occurred about 23–25 hr. after the onset of oestrus. They were inseminated 14–39 hr. after the end of oestrus and about 9–32 hr. after the probable time of ovulation, and none conceived. In grade Merino sheep sperm can retain its fertility for nearly 24 hr. and the findings of other workers indicate that sperm are capable of reaching the upper end of the Fallopian tubes fairly quickly. It therefore seems that the period for which the ovum of the ewe can be fertilized must be brief.

It is reported in this paper that spermatozoa from a Merino ram are capable of retaining their fertilizing capacity in the genital tract of the ewe for 22½ hr., the maximum period investigated in this experiment. Quinlan *et al.* have studied the vitality of spermatozoa of Merino rams in Merino ewes. In the vagina the majority of the spermatozoa are non-motile after 12 hr. Living spermatozoa were found in the cervix up to 48 hr. after coitus and it is believed that the cervix acts as a reservoir for spermatozoa awaiting the availability of the ovum.

These workers, assuming that the ovum is available for fertilization between the 36th and 40th hour (the interval after the onset of oestrus when ovulation is believed to occur), plus a period of a few hours taken by the spermatozoa to reach the Fallopian tube, state that spermatozoa are definitely capable of fertilization for 36–42 hr. after being deposited in the vagina. Green & Winters (1935), stated that spermatozoa do not live more than about 24 hr. in the genital tract. According to Polovceva *et al.* (1938), the average duration of survival of spermatozoa in the female genital tract may be estimated at 34–36 hr., but in several instances eggs were fertilized which had ovulated 40–50 hr. after insemination. Kelley (1937) found that the fertilizing power of spermatozoa from even the most fertile rams reached the threshold of infertility at approximately 34 hr. after copulation. The fertilizing power of the ejacula from the majority of the Merino rams had a shorter duration than that of the ejacula from the Dorset rams. In Kelley's (1937) experiment the limit of fertilizing power in hours post-coitus was 24 hr. for Merino rams. Further, there is evidence that the fertilizing power of the ejacula of Merino rams becomes reduced, if not lost, 10 hr. after coitus.

There is some difference of opinion on the time taken by ram spermatozoa to traverse the genital tract and reach the upper extremity of the Fallopian tubes. These times, following coitus, found by different workers are, (1) within 6 hr. (Quinlan *et al.* 1932), (2) about 5 hr. (Kelley & Dumaresq, 1936), (3) about 5 hr. (Green & Winters, 1935), (4) from 30 min. to 7 hr. 7 min. (Phillips & Andrews (1937), and (5) about 5 hr. in Merino ewes (Kelley, 1937).

Assuming that spermatozoa are capable of retaining their fertilizing capacity in the ewe for a certain period of time, it does not follow that they will be equally fertile throughout this period, although the few results given in this paper do not indicate any falling off in fertility after 22½ hr. It is probable, however, that fertility tends to diminish according to the length of stay in the genital tract, as is indicated by Kelley (1937). The vitality and fertilizing capacity of spermatozoa in the genital tract of the ewe are probably dependent on a number of factors which include the initial vitality and the capacity of spermatozoa for retaining this vitality and the condition of the genital tract of the ewe. It would appear from the work of Kelley that breed differences exist in the length of time for which spermatozoa retain their fertilizing capacity.

Number of inseminations. It is somewhat difficult to come to a definite conclusion about the number of inseminations required per heat period in grade Merinos. In actual practice the object is to ensure that there are available at the time of ovulation a sufficiency of spermatozoa of high vitality, and this, in view of the work already discussed could probably be attained by the introduction of spermatozoa at say, 5–6 hr. before the time of ovulation. Kelley (1937) comes to a similar conclusion. The effective life of spermatozoa in the ewe is, however, probably considerably longer than this, but it has not been accurately determined and the introduction of spermatozoa earlier than 5–6 hr. before the time of ovulation probably gives good results. Warbritton *et al.* (1937) stated that of three periods of insemination in the ewe, 12 hr. before ovulation was most desirable; for many ewes this meant breeding 10–18 hr. after the onset of oestrus. Kardymovic *et al.* (1934) found that the optimum time was 18–26 hr. after the onset of oestrus (the duration of oestrus not stated); at this time the percentage of conceptions was 84·8, and at 26–42 hr. after the beginning of oestrus the percentage of conceptions had fallen to 48, presumably due to a number of ewes having gone off heat before being inseminated. Zajac (1935) found that the optimum time was 24 hr. from the beginning of heat, but even at 48 hr. after the beginning of heat 77 % of ewes conceived. Since the time of ovulation is usually related to the end of oestrus, the optimum time for insemination depends on the duration of oestrus and this will vary in different breeds and under different conditions. Kelley (1937), for example, obtained a higher percentage of conceptions with matings within a maximum of 4 hr. from the onset of oestrus, in Merino ewes, than in Dorset ewes, which had a longer oestrous period.

When rams are run with the ewes, a ewe is probably served several times during one heat period. However, a single service has given similar

results to two or more services during the one heat period (unpublished results). Kelley (1937) found a difference in favour of several services. On the whole, in artificial insemination experiments at Naivasha, somewhat better results have been obtained by giving two or more inseminations in the one heat period, but the inseminations were not always carried out at the same season of the year and the results are not therefore strictly comparable. According to Milovanov (1934) the results of repeated inseminations have been excellent in some cases and negative in others. It is stated that it will be effective if the ewe is genetically capable of producing more than one ovum; if environmental conditions are favourable; if the interval between ovulations is long; and if the vitality of the spermatozoa is low. From experiments on 1100 ewes, Kirillov (1938) concluded that all ewes should be tested for heat twice a day, morning and evening and only ewes with a heat period of more than 24 hr. should be inseminated twice (the duration of heat in these ewes was under 24 hr. in 47 % of ewes, and 24–36 hr. in 45 % of ewes). It is stated that inseminating ewes with heat periods lasting over $1\frac{1}{2}$–2 days, three or four times, does not raise the rate of lambing.

In grade Merino ewes at Naivasha, the mean duration of oestrus (1261 periods) was 26 hr. (author, unpublished results). In 1936, about one-third of the ewes began their heat periods between 6 and 9 a.m., another third between 9 a.m. and 3 p.m., and the remainder between 3 p.m. and 6 a.m. Since 1937 the usual practice has been to pick out ewes on heat, (1) in the morning and to inseminate them about 4 p.m. that day and again about 9 a.m. the following morning, and (2) during the day and inseminate them also at 4 p.m. that day and again about 9 a.m. the following morning. Thus, of ewes picked out in the morning about one-half have probably come on heat that morning and about one-half since the previous afternoon. For the former, the maximum duration of oestrus up to 4 p.m. that day would be 10 hr., and up to 9 a.m. the following morning 27 hr., and for the latter 25 and 42 hr. at these times. For ewes picked out during the day from 8 a.m. to 3 p.m., the maximum duration of oestrus at the times of insemination would be 8 and 25 hr. The upper limit for the interval since the onset of heat in the morning ewes is too high, for many ewes will have gone off heat by the time of the second insemination. For the day ewes, the upper limit is more satisfactory and it might therefore be expected that the day ewes would give better results. In the 1939 experiment, the differences were not great, the day ewes giving 32·4 % of conceptions and the morning ewes 29·2 %. In general, the procedure would seem to be appropriate for the majority of ewes, though the upper limit at the time of the second insemination may

be rather on the high side for some of the ewes. Since, however, as good results have been obtained with a single as with a double insemination in the one heat period, this question requires further investigation, and it may prove that in grade Merino ewes a single insemination at a favourable period of the year would be adequate.

Dilution. The extent to which sperm may be diluted depends mainly on the effect of the diluent on the spermatozoa and on the number of spermatozoa required for fertilization. Walton (1927) found that in the rabbit, fertility is influenced by the number of spermatozoa introduced into the vagina. The optimum dilution for various ram sperm diluents has been worked out. Diluent GPS-2, for example, which has been used in all the Naivasha experiments, has an optimum dilution of $\times 8$, but for practical purposes the addition of 1–3 parts diluent is advised (Milovanov, 1934). There is little information about the density of spermatozoa required for fertility in sheep. Milovanov *et al.* (1937) give the standard number of spermatozoa required for each insemination as 500×10^6, but state that this varies from 1200×10^6 to 75×10^6 according to the resistance of the spermatozoa. (This number, however is for vaginal insemination; cervical insemination requires less.)

In pure-bred Merino rams in Kenya, the average number of spermatozoa per c.mm. is 2·5 millions and the average volume of the ejaculate is 0·72 ml., i.e. an average ejaculate contains about 1800 million spermatozoa. With the addition of 3 parts diluent to such an ejaculate, a dose of 0·2 ml. would contain 125 million spermatozoa, which might possibly be on the low side. In Naivasha experiments, there has not been on the whole, great differences between the use of diluted and undiluted sperm, but ordinary service has given a higher percentage of conceptions than the use of undiluted sperm: 70 % conceptions were obtained from hand service compared with 47 % from undiluted sperm in August 1936 (author, unpublished results). It is possible that this difference is due to the greater density of sperm introduced into the genital tract of the ewe in ordinary service rather than to better synchronization between the time of introduction of sperm and the time of ovulation, since both lots of ewes having been picked out in the morning were probably inseminated at approximately the same period of heat. Demidenko *et al.* (1933) also found a higher percentage of conceptions from ordinary mating, and little difference between sperm used diluted up to $\times 4$, and undiluted.

General. With ordinary service a higher percentage of conceptions is obtained per heat period than with artificial insemination. Therefore, provided a high percentage of ewes come on heat, it is easier to get a satisfactory lambing from ordinary service, even in less favourable

seasons. With artificial insemination, when conditions are unfavourable to reproductive activity during the breeding season, ewes which fail to 'hold' take a long time to return to the ram. Their chances for re-insemination are consequently much reduced and a poorer lambing results. When conditions are favourable, the smaller percentage of conceptions at the first heat period from artificial insemination compared with ordinary service, does not prevent a good lambing, for the ewes return to the ram at the normal cyclical interval and can be re-inseminated twice or three times during the breeding season. It also seems, though the evidence is as yet somewhat inconclusive, that during a favourable season the incidence of conceptions per heat period from artificial insemination is higher than at other times of the year. The first essential for successful artificial insemination of high grade Merino sheep in Kenya is therefore to choose the best period of the year for the breeding season. At Naivasha this seems to be in the earlier part of the year.

The somewhat better results from two or more inseminations per heat period may be due to better synchronization between the times of introduction of sperm and the time of ovulation, but this is also as yet undecided, for single inseminations during certain seasons have given equally good and better results, and this may be due mainly to seasonal factors rather than to other reasons. Since a single service has given better results than a single insemination it would also seem that more attention should be paid to the number of spermatozoa introduced into the cervix at each insemination.

Ram. The fertility of rams varies considerably even when the character of the sperm is of the highest degree, according to present criteria. This constitutes one of the most important problems in artificial insemination. In the absence of a reliable criterion it is possible to obtain information on the actual fertility of rams by allowing them to serve a number of ewes before the breeding season and seeing how they 'settle' them. Uncompleted experiments indicate that there may be seasonal variations in reproductive capacity in the ram.

Summary

The results obtained from artificial insemination of high grade Merino sheep in Kenya are given and discussed in relation to the season of the year, time of insemination during oestrus, the number of inseminations in each oestrous period and the degree of dilution of sperm.

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THE HYDROGEN-ION CONCENTRATION OF THE SEMEN OF THE BULL

By J. ANDERSON

THE HYDROGEN-ION CONCENTRATION OF THE SEMEN OF THE BULL

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(With Five Text-figures)

Within recent years much work has been done on the semen of the domesticated animals, particularly in connexion with the widespread application of artificial insemination. The evaluation of semen at present includes the determination of the volume of the ejaculate, the degree of motility of spermatozoa, the number of spermatozoa per c.mm., the total number of spermatozoa per ejaculate, the type and number of abnormal spermatozoa, and the respiratory rate of spermatozoa. Greater or lesser stress is laid upon one or other of these evaluations by different workers. No single criterion has, however, been found adequate to determine small differences in fertility (Milovanov, 1936; Edwards & Walton, 1939; Anderson, 1940, 1941). Apart from the respiratory rate of spermatozoa, which has been found to give a better indication of fertility than any of the other measurements (Edwards & Walton, 1939), it seems that the best evaluation that can be made at present is that based on a number of the criteria. From the work described in this paper and that of others, it appears that the determination of the hydrogen-ion concentration of bull semen, perhaps either by itself or together with one or other of the above measurements, will provide an estimate of value.

One of the earlier estimations of the *p*H of bull semen was made by Webster (1932), who found that almost all samples lay between 7·0 and 7·5. Later investigations by Webster (1938), using the Cambridge potentiometer, indicate a *p*H range of 6·00–7·50. According to Milovanov (1934), the *p*H is usually acid, 6·5–6·8, sometimes even 5·5, but with an increase in the amount of the accessory secretions may become neutral or even alkaline, 7·0–7·5. Davis (1938) found that the *p*H varied widely as between semen collected by massage of the ampullae and seminal vesicles, and the artificial vagina; by the former method, which yields a relatively greater amount of accessory secretions, a *p*H of 7·5–8·0 was not uncommon, while for the latter method the *p*H was ordinarily below 7·0. Sergin (1935), using a quinhydrode electrode, obtained a *p*H of 6·74 and Hatziolos (1937) a *p*H range of from 6·39 to 7·81, with a mean of $6{\cdot}897 \pm 0{\cdot}107$. Davis & Williams (1939), using a quinhydrode gold electrode, obtained a *p*H which ranged from 6·18 to 8·31, with a mean value of 6·99. A significant inverse relationship between the *p*H of ejaculates and the number of spermatozoa per c.mm., in that the greater the concentration of spermatozoa, the lower the *p*H, has been noted (Schneerson, 1936; Davis & Williams, 1939). Davis & Williams also noted a significant negative correlation between the *p*H and volume of the ejaculate and *p*H and motility of spermatozoa. An inverse correlation between *p*H and concentration of spermatozoa has also been noted for ram spermatozoa (McKenzie & Berliner, 1937). Increasing the temperature of the scrotum of the ram caused the semen to become more alkaline (Gunn, 1936; McKenzie & Berliner, 1937). The semen of the ram after this heat treatment of the scrotum is thin and watery and the change in *p*H is apparently due to the decrease in the number of spermatozoa.

The reaction of the semen became neutral or alkaline in sterile rams (Webster, 1934–5; McKenzie & Berliner, 1937).

The *p*H of the epididymal contents is about 6·5 and that of the testis itself varies from 7·19 to 7·37 (Milovanov, 1934). This acid reaction in the epididymis has been shown by Lanz (1929) to be due to the secretory activity of the epididymis, which is influenced by the hormonal secretion of the testes. Removal of both testes created an alkaline reaction in the epididymis.

There is an increase in the acidity of semen on storage (Milovanov, 1934), and this appears to be mainly due to an accumulation of lactic acid (Bernstein & Slovohotov, 1933; Sergin, 1935). Hatziolos (1937) indicated that there might be a small positive correlation between *p*H and duration of life of spermatozoa. Schneerson (1936) obtained best survival of bull spermatozoa at a *p*H of 6·4; the survival at a *p*H of 6·8–7·0 was only a quarter of the maximum. Davis & Williams (1939), who also found that the *p*H became lower on storage, noted a slow but steady decline for all temperatures, except for 70° F. when the decline was rapid. Temperatures of 35 and 40° F. were superior to 50° F. in slowing down the decline in *p*H. Davis (1938) observed that a high *p*H was not conducive to preservation. A lowering of *p*H with increased temperature has also been noted for human spermatozoa (Zagami, 1938).

MATERIAL AND METHODS

The semen was collected with the artificial vagina, following the method of Walton (1936) and Russian workers. This method has already been described in full (Anderson, 1939). For storage, semen was placed in pyrex test-tubes, the tube filled with liquid paraffin (Walton, 1936), corked and sealed with candle wax. The tube was then wrapped in cotton-wool and placed in a thermos flask at a temperature of 8–10° C. The cooling was thus sudden. The maintenance of the temperature in the flask was best accomplished by keeping the flask in a refrigerator. When necessary the temperature in the flask was adjusted twice daily. The *p*H of the semen was determined with a glass electrode (Beckman *p*H meter). The estimation of the motility, which was expressed as the percentage of spermatozoa showing very active movement, was largely subjective, but since the estimations were all made by one person, they afford a relative indication of the differences between ejaculates. No ejaculate was stored unless it showed an initial motility of 80% or over. Since there was some variation in the initial degree of motility, the motility of ejaculates in Table 5 was expressed as 100% on collection and as a percentage of this value at 24 hr. and later.

RESULTS

The mean *p*H of 221 ejaculates of clinically normal bulls was $6{\cdot}73 \pm 0{\cdot}020$ ($s = 0{\cdot}300$). Analysis of variance (Snedecor, 1938) of the semen of eleven fertile bulls on the Experimental Station, during a period of 6 months from August 1940 to January 1941, showed that the individual differences were just significant (Table 1). The range in the *p*H of the semen of these bulls was from 6·36 to 7·61. Of the 159 ejaculates from these eleven bulls, twenty-five (15·7%) had a *p*H greater than 6·91 and ten ejaculates (6·3%) with this *p*H were from one bull.

Table 1. *Analysis of variance in pH of semen of bull*

Source of variation	D.F.	Sums of squares	Mean square
Bull	10	1·5388	0·1539
Month	5	0·5535	0·1107
Bull-month interaction;	50	2·1292	0·0427
Within subclasses	93	7·3685	0·0792
Total	158	11·5900	0·3865

Significant. Bull: $F = \frac{0{\cdot}1539}{0{\cdot}0792} = 1{\cdot}9431$ just exceed 5% point of 1·92 for 10 and 93 D.F.

Not significant. Month: $F = \frac{0{\cdot}1107}{0{\cdot}0792} = 1{\cdot}3977$. 5% point for 5 and 93 D.F. is 2·30.

A monthly variation in the *p*H of the semen of these bulls was noted as follows: August, 6·84; September, 6·70; October, 6·67; November, 6·61; December, 6·70; and January, 6·70. This seasonal variation was not statistically significant (Table 1), but it is possible that it might prove to be so over a longer period.

Table 2. *Correlation and regression of bull semen*

	No.	Mean	*s*	*r*	Regression coefficient	S.E. O.E.	*t*
*p*H and concentration	171	6·722 ± 0·019 648·54 ± 34·30	0·252 448·69	−0·3810	$E = 6{\cdot}858 - 0{\cdot}00021X$	0·233	5·25
*p*H and volume	195	6·708 ± 0·019 3·53 ± 0·135	0·270 1·887	−0·4206	$E = 6{\cdot}920 - 0{\cdot}06006X$	0·244	6·46
*p*H and motility	221	6·730 ± 0·020 73·12 ± 1·80	0·300 26·74	−0·5350	$E = 7{\cdot}170 - 0{\cdot}0060X$	0·2535	9·52

A highly significant linear regression of the *p*H on the concentration of spermatozoa, on the volume of the ejaculate and on the motility of the spermatozoa has been noted (Table 2; Figs. 1–3). The more acid the *p*H the greater was the concentration of sperma-

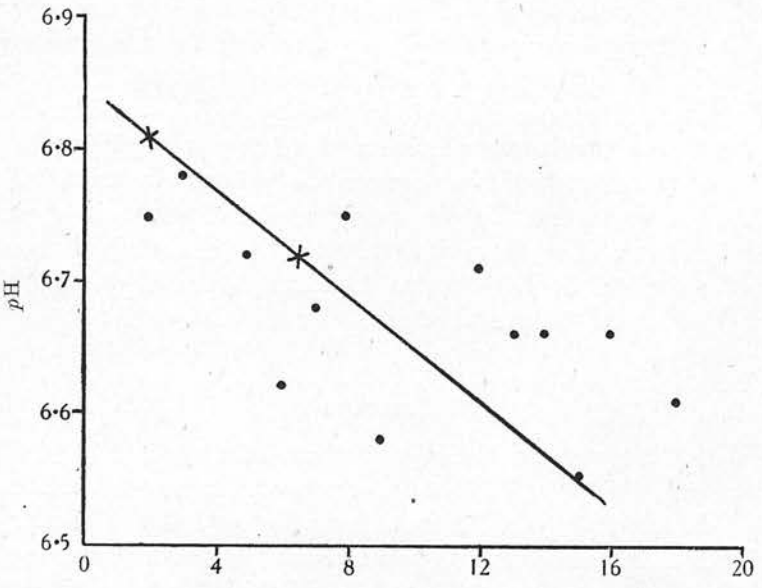

Fig. 1. Regression of *p*H on concentration of spermatozoa. The black dots indicate the mean *p*H position of points at the centre of 100,000 spermatozoa classes.

tozoa, the higher the degree of motility and the larger the volume of the ejaculate. There were, however, exceptions to this inverse relationship between the *p*H and concentration of spermatozoa and the degree of motility. Out of a total of 221 ejaculates, twelve ejaculates had a *p*H in the range 6·31–6·90, associated with a mean motility of 38% (range 10–60%) and a mean number of spermatozoa of 274,000 per c.mm., and twenty-two ejaculates had a *p*H of 6·91–7·20, associated with a mean motility of 76% (range 70–100%) and a mean number of spermatozoa of 436,000 per c.mm. In no instance has a high motility been found with a *p*H greater than 7·60.

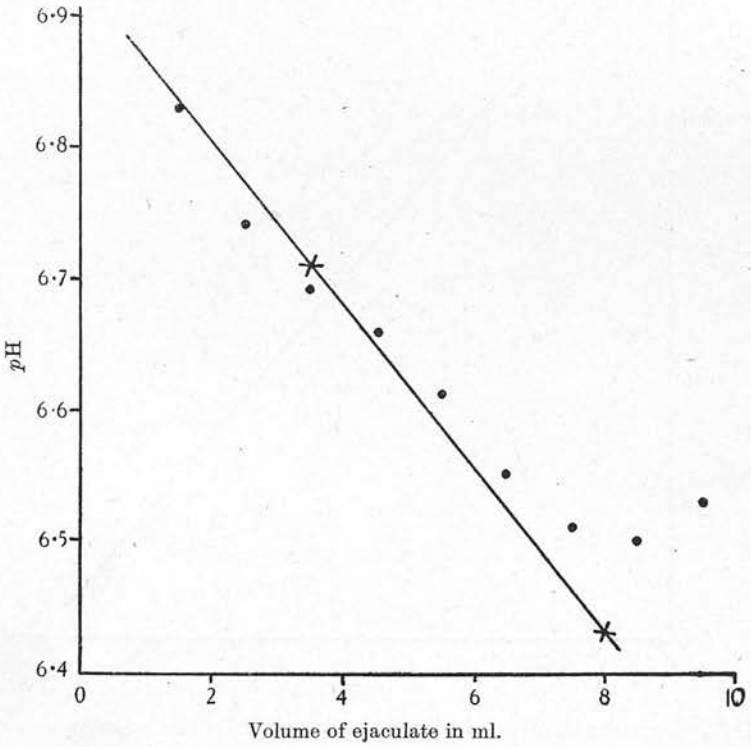

Fig. 2. Regression of *p*H on volume of ejaculate. The black dots indicate the mean *p*H position of points at the centre of 1 ml. classes.

On thirty-two occasions two ejaculates were collected, the second within a few minutes of the first. The *p*H of the first ejaculates averaged 6·73 and the second 6·67. Second ejaculates had also a larger volume, a higher motility and a greater concentration of spermatozoa.

Ejaculates which kept well were recognized as those which had a motility of 70% and over at 24 hr. or later. Sixty-three ejaculates which kept well had a mean *p*H of $6{\cdot}573 \pm 0{\cdot}016$ ($s = 0{\cdot}129$), and seventy-three ejaculates which kept badly had a mean *p*H of $6{\cdot}739 \pm 0{\cdot}022$ ($s = 0{\cdot}194$). The difference between these two means is highly significant ($t = 5{\cdot}9$). Sixteen of the ejaculates which kept badly had an initial *p*H of 6·91 or higher. This *p*H, therefore, appears to be about the borderline, in that none of the ejaculates of this *p*H or higher kept well. The other fifty-seven ejaculates which kept badly had an initial *p*H of 6·90 or lower (mean $6{\cdot}646 \pm 0{\cdot}017$, $s = 0{\cdot}132$). The difference between the mean *p*H of the sixty-three ejaculates with an initial *p*H of 6·90 or lower which kept

well and the mean *p*H of the fifty-seven ejaculates, also within initial *p*H of 6·90 or lower, which kept badly is highly significant ($t = 3{\cdot}2$). Thus, within the limits of these observations, the more acid the semen on collection the better it retained its motility on storage.

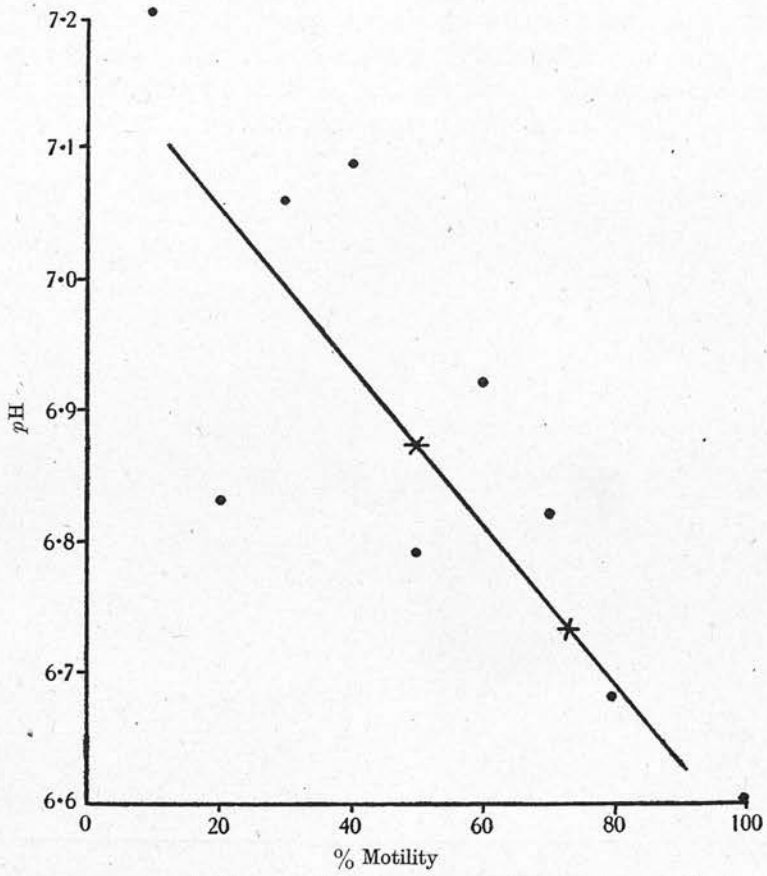

Fig. 3. Regression of *p*H on motility of spermatozoa. The black dots indicate the mean *p*H position of points at the centre of 10% motility classes.

There were three main types of changes in *p*H during storage in ejaculates with a low initial *p*H, i.e. below 6·90, (*a*) the *p*H decreased steadily throughout the period of storage, (*b*) the *p*H increased for 24 hr. and then decreased for the remaining period of storage, and (*c*) the *p*H increased for 48 hr. or longer (Table 3). 88·2% of the 136 ejaculates had a low initial *p*H, and of these 120 ejaculates, about 45% decreased steadily in *p*H, about 46% increased in *p*H for 24 hr., and the remaining 9% of ejaculates increased steadily in *p*H during the period of storage.

Table 3. *Mean change in p*H *on storage*

	No. of ejaculates	*p*H at hours						
		0	24	48	72	96	120	144
Initial *p*H low	54	6·57	6·44	6·30	6·26	6·22	6·18	6·03
	55	6·61	6·85	6·61	6·53	6·43	6·14	—
	11	6·56	6·78	6·86	6·90	—	—	—
Initial *p*H high	9	7·00	6·87	6·76	6·83	—	—	—
	7	7·01	7·32	—	—	—	—	—

For the 120 ejaculates with an initial *p*H below 6·90 the data were examined to see if there was any relationship between the initial *p*H and the decrease and increase that occurred in the *p*H during storage. The mean initial *p*H of the fifty-four ejaculates in which the *p*H decreased during storage was $6{\cdot}570 \pm 0{\cdot}019$ ($s = 0{\cdot}139$), and of the sixty-six ejaculates in which the *p*H increased during storage was $6{\cdot}614 \pm 0{\cdot}018$ ($s = 0{\cdot}147$). The difference between these means was not, however, significant. When, however, the data were examined in more detail (Table 4), it was clear that as the initial *p*H decreased from 6·90, the percentage of ejaculates in which the *p*H decreased during storage became higher and the percentage of ejaculates in which the *p*H increased during storage became lower and vice versa. These changes were most marked below 6·50 and above 6·71.

Table 4. *Initial p*H *and increase or decrease in p*H *during storage*

*p*H	No. of ejaculates	% of ejaculates	
		*p*H decreased	*p*H increased
6·81–6·90	7	28·6	71·4
6·71–6·80	24	37·7	62·3
6·61–6·70	24	50·0	50·0
6·51–6·60	34	41·2	58·8
6·41–6·50	22	54·5	45·5
6·31–6·40	9	55·5	44·5
6·31–6·70	89	48·3	51·7
6·71–6·90	31	35·5	64·5

These changes in *p*H had an important effect on the motility of stored spermatozoa (Table 5, Fig. 4). The motility was best retained when the *p*H decreased throughout the

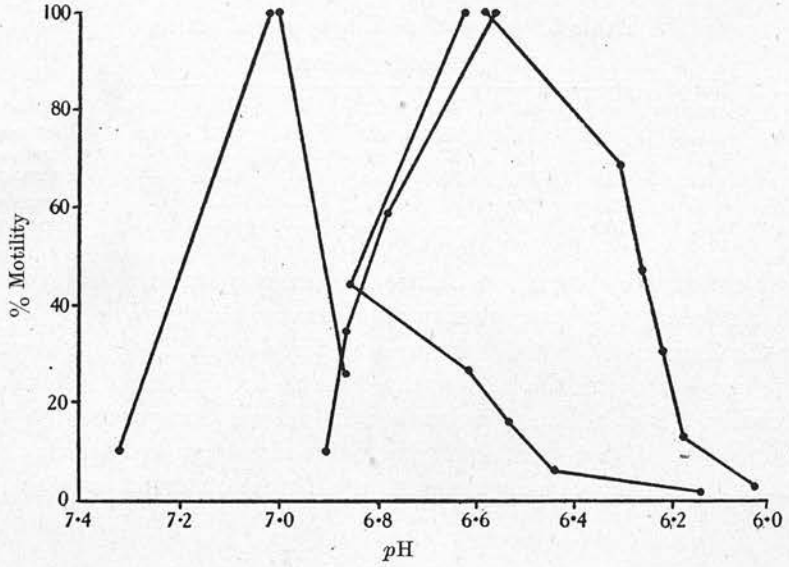

Fig. 4. *p*H and % motility of semen stored undiluted. The intervals between the black dots represent periods of 24 hr.

period of storage. When the *p*H either (*b*) increased for 24 hr. and then decreased or (*c*) increased steadily the motility was not well retained; with the latter (*c*) the motility at 24 hr. was better than for (*b*) and the *p*H of (*c*) at 24 hr. was lower than that of (*b*). Motility in (*b*) was retained longer than in (*c*), and this occurrence was probably associated with the differences in *p*H. The percentage of ejaculates with high motility (70%

and over) in relation to *p*H is shown in Fig. 5. These average differences show that semen which retained its motility well become increasingly acid and that any decrease in the acidity was harmful.

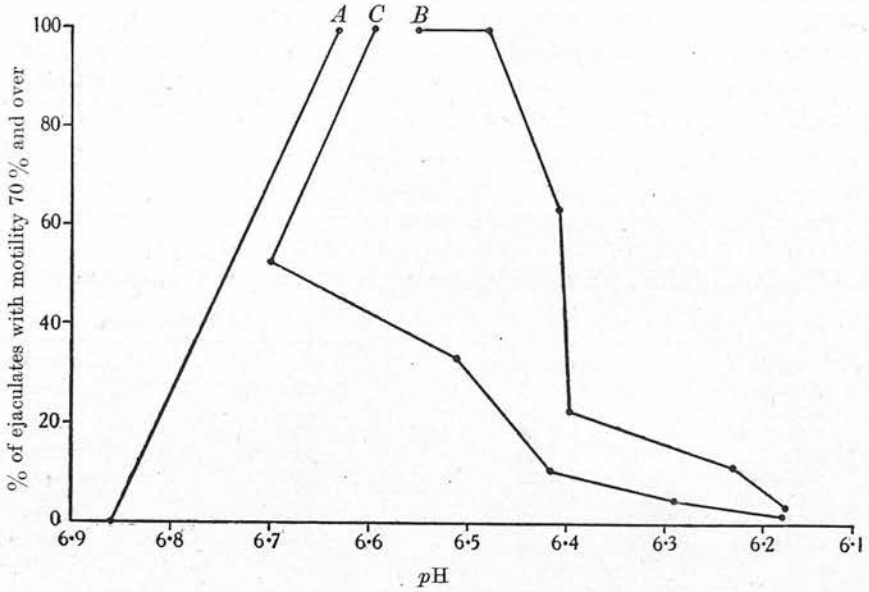

Fig. 5. *p*H and % ejaculates. *A*, ejaculates with poor motility on storage. *B*, ejaculates with good motility on storage. *C*, all ejaculates.

Table 5. *p*H *and motility of stored semen*

	No. of ejaculates	% motility at hours							Change in *p*H
		0	24	48	72	96	120	144	
Initial *p*H low	54	100	84	68	47	30	13	3	Decreased
	55	100	44	27	16	6	—	—	Increased for 24 hr.
	11	100	58	35	10	—	—	—	Increased steadily
Initial *p*H high	9	100	26	—	—	—	—	—	Decreased
	7	100	9	—	—	—	—	—	Increased

There was considerable variation between different ejaculates and different groups of ejaculates in relation to keeping quality of semen and changes in *p*H (Tables 6 and 7). Of the sixty-three ejaculates which kept well, in forty-seven (74·6 %) the *p*H decreased steadily, in twelve ejaculates the *p*H increased for 24 hr. and in four ejaculates the *p*H increased steadily. Thus, although a decrease in *p*H on storage was the predominant feature of semen which kept well, some ejaculates also kept well which showed a rise in *p*H for 24 hr. or longer. Similar changes on *p*H were observed in semen which kept badly (Table 3).

Table 6. *Low initial p*H *and variation in keeping quality*

Keeping quality	No. of ejaculates	% motility at hours						
		0	24	48	72	96	120	144
Kept well	47	91	83	68	50	31	15	3
	12	88	76	69	52	21	12	1
	4	85	73	55	23	—	—	—
Kept badly	7	91	40	21	13	7	—	—
	43	80	32	12	5	4	—	—
	7	77	39	17	4	—	—	—

Table 7. *Low initial pH and change in pH in relation to keeping quality of semen*

Keeping quality	No. of ejaculates	pH at hours 0	24	48	72	96	120	144
Kept well	47	6·56	6·35	6·30	6·30	6·21	6·18	6·02
	12	6·54	6·71	6·53	6·45	6·31	6·14	5·99
	4	6·48	6·48	6·77	6·90	—	—	—
Kept badly	7	6·60	6·50	6·27	6·15	—	—	—
	43	6·63	6·84	6·66	6·59	6·54	—	—
	7	6·62	6·85	6·98	6·75	6·74	—	—

Examining these data in a different way, of the fifty-four ejaculates in which the *p*H decreased steadily during storage, forty-seven (87 %) kept well. On the average the initial *p*H of the ejaculates that kept well was lower than that of those which kept badly and the increase in acidity was more marked in the former than in the latter. Of the fifty-five ejaculates in which the *p*H increased for 24 hr. and then decreased for the remaining period of storage, only twelve (21 %) kept well. The initial *p*H of the ejaculates that kept well was lower than that of those which kept badly and there was also a less marked decrease in acidity at the 24th hour in the former. Of the ejaculates in which the *p*H increased for 48 hr. or longer, only four kept well. Here again the initial *p*H of the ejaculates which kept well was lower than that of the ejaculates that kept badly and the decrease in acidity at the 24th and 48th hours was less marked in the former.

DISCUSSION

Observations by other workers on the semen of the bull and on the inverse relationship between the *p*H and the concentration of spermatozoa, the degree of motility and the volume of the ejaculate have been confirmed. This significant negative correlation should prove useful for the evaluation of semen. The determination of the *p*H will give an indication of the concentration as well as of the motility of the spermatozoa. There were, however, about 15 % of ejaculates for which this negative correlation did not hold and for which *p*H alone did not give a true evaluation of the semen. The determination of both *p*H and motility, however, as Davis & Williams (1939) suggested should form a sound basis for evaluation of semen.

One of the great difficulties in storing bull semen in vitro has been the great variation between different ejaculates, in the period for which a high motility (i.e. 70 % and over) was retained. When semen was stored undiluted after sudden cooling to 8–10° C. many ejaculates commonly had a low motility after 24 hr. In practice with a large number of ejaculates, only about three-quarters showed a high motility after 24 hr. and about one-third after 48 hr. In the present series of 136 ejaculates, only sixty-three retained a high motility after 24 hr. Any characteristics of semen, therefore, which will indicate, at the time of collection, the potential survival capacity of the semen will have considerable value. The present observations show that the estimation of the *p*H of semen at the time of collection should help to some extent, at least, to indicate which ejaculates will survive best.

Davis (1938) noted that high *p*H was not conducive to preservation and this observation is confirmed. None of the ejaculates with an initial *p*H of 6·9 or higher have shown a high degree of motility after 24 hr. In extension of this observation it has been

shown that the more acid the semen on collection the better a high degree of motility was retained.

Whatever the initial *p*H of the semen, the *p*H during storage may either decrease, increase for 24 hr., or increase for 48 hr. or longer. Motility was best retained when the *p*H decreased steadily throughout the period of storage. When the *p*H increased for 24 hr. or longer the motility was not so well retained. The more acid the semen was on collection and the less it moved in an alkaline direction during storage the better was the motility retained. A move in the alkaline direction was more harmful for ejaculates with a higher initial *p*H than for those with a lower initial *p*H. The initial *p*H and the degree and direction of the change in *p*H during storage were therefore of primary importance for the retention of high motility in stored semen.

It appears that the change in *p*H in an alkaline direction is affected to some extent by the initial *p*H (Table 4). Below an initial *p*H of 6·50 a higher percentage of ejaculates decreased in *p*H on storage and above 6·71 a smaller percentage decreased in *p*H during storage. Likewise, below 6·50 a smaller percentage of ejaculates increased in *p*H during storage and above 6·71 a higher percentage of ejaculates increased in *p*H during storage. The more acid the *p*H on collection the less likely was a change in the alkaline direction during storage.

The determination of the *p*H of semen at the time of collection therefore provides a valuable estimate of the relative potential survival capacity of different ejaculates. An alkaline reaction in the semen of the ram was associated with sterility (Webster, 1934–5; McKenzie & Berliner, 1937) and Webster (1938) found that distinctly acid ejaculates in the ram were of high fertility, and that as the degree of acidity decreased towards the neutral point so did the fertility. A limited number of *p*H testes on the semen of bulls by Webster (1938) gave apparently definite indications of an identical correlation between *p*H and fertility, as in the ram, but probably the critical range may be rather higher. It is tentatively suggested that the probable fertile *p*H range is from 6·00 to 7·5 with samples between 7·0 and 7·5 of very doubtful low fertility. The realization of the importance of the *p*H of semen opens possibilities of improving the quality of the semen through measures aiming at increasing the acidity of the semen in vitro or in vivo and experiments on these lines are in progress.

Summary

1. The mean *p*H of 221 ejaculates of clinically normal bulls was 6·73 ± 0·020. Significant differences were noted between different bulls.

2. There were highly significant negative correlations between the *p*H of the ejaculates and the concentration of spermatozoa, the volume of ejaculate and the motility of the spermatozoa.

3. The more acid the semen was on collection, the better was the motility retained on storage. Semen which retained its motility well became increasingly acid during storage. A change in the *p*H in the alkaline direction during storage had an adverse effect on motility. The more acid the *p*H on collection the less likely was a decrease in acidity during storage.

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THE PERIODICITY AND DURATION OF OESTRUS IN ZEBU AND GRADE CATTLE

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(With Twelve Text-figures)

In a previous investigation on the periodicity and duration of oestrus in zebu cattle in Kenya (Anderson, 1936), it was found that oestrus was of very short duration, while the cycle was fairly normal in length. Since the experimental animals were existing on pasture which was of poor nutritive value, being particularly low in protein and phosphorus, it was desirable that these observations should be repeated under optimum nutritional conditions. This has now been done, and similar observations have also been made on high-grade cattle for which there was no previous data.

One of the main points of interest is the investigation of seasonal changes in sexual function and their possible correlation with climatic conditions. The existence of such seasonal changes, as was shown in the first experiment, is confirmed by the present data, which present and illustrate a relationship between certain climatic factors, such as temperature and sunshine, and sexual function.

PHYSIOGRAPHICAL CONDITIONS

The Experimental Station, Naivasha, is situated in the Rift Valley District of Kenya, latitude 0·43′ S., longitude 26·26′ E., at an altitude of 6231 ft. above sea-level. The average annual rainfall is about 20 in.

The meteorological data which were recorded included the maximum and minimum temperatures, the wet- and dry-bulb temperatures and the number of daily hours of sunshine. During the period of the experiment the recordings were made once daily, at 8.30 a.m. The wet- and dry-bulb temperatures for the period September 1941 to April 1942 are included to illustrate the changes that occur during the day in temperature and humidity. The number of daily hours of sunshine was recorded on a Jordan sunshine recorder. There are no data for ultra-violet light, but Baker (1937) points out that where the sun is overhead twice during the year, which it is on the equator at the vernal equinox (21 March) and the autumnal equinox (23 September), there are two periods of maximum ultra-violet light.

The humidity of the air can be variously expressed, but commonly the relative humidity, which represents the percentage degree of saturation of the air, is used. Baker (1936) stated that the expression of atmospheric humidity in terms of saturation deficiency, instead of relative humidity, has the advantage that figures tend to be proportional to the drying power of the atmosphere, whatever the temperature may be. The saturation deficiency is expressed in grams per cubic metre (i.e. the number of grams of water that one would have to add to a cubic metre of air to saturate it, the temperature remaining constant). Baker stated that if two lots of air have the same saturation deficiency, similar objects would tend to dry at the same rate in both of them, although the temperature of the two may be different; if one lot has twice the saturation deficiency of the other, similar objects would tend to dry twice as quickly as in the first. The saturation deficiency represents the rate of evaporation into still air. Belham (1937), however, questions whether the saturation deficiency has more claim to consideration as a measure of evaporation than the depression of the wet bulb. Saturation deficiency and relative humidity have both been used in this study, and as both expressions of humidity show similar seasonal variations there does not seem to be any greater advantage attached to either for present purposes.

Rainfall. The annual rainfall in 1936, 1937, 1938 and 1939 was 20·24, 33·16, 20·37 and 24·54 in. respectively. The distribution of the rainfall varied considerably from year to year (Fig. 1), but in general the main rainy season was in March, April and May and the shorter rains in the October-November period.

Temperature. The mean daily maximum temperature from August 1936 to December 1939 showed considerable variations (Fig. 1). The coldest month was May 1938 (63·3° F.) and the warmest, August 1939 (83·3° F.). The mean monthly maximum temperatures were fairly uniform, varying from about 71 to 78° F., as were the means of the monthly maximum and minimum temperatures, which varied from 60·2° F. in June to 63·3° F. in March. The lowest mean daily minimum temperature was in September 1937 (42·9° F.) and the highest in April 1937 (53·5° F.). The mean monthly minimum temperatures for 1937 to 1939 inclusive varied from 43·2° F. in September to 51·1° F. in April.

The diurnal range in temperature, which was

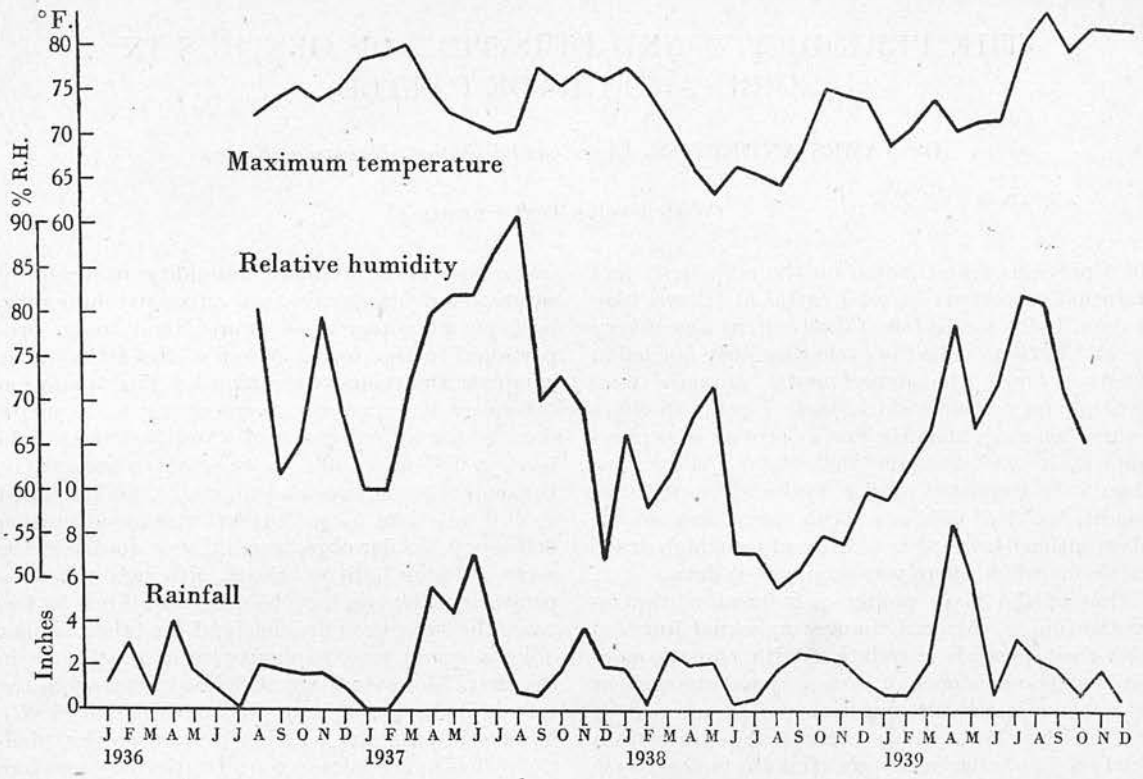

Fig. 1. Meteorological data.

marked (range 19·3° F. in April to 30·6° F. in September) is averaged for the period 1937–9 inclusive in Fig. 2. This range was greater than 24° F. for the months of January, February and March and for August to December. The extreme monthly figures for the difference between mean daily maximum and minimum temperatures was 12·4° F. in May 1938 and 36·2° F. in August 1939.

Dry-bulb temperatures taken throughout the day are not available for the period of the experiment, but they are shown for the period September 1941 to April 1942 inclusive, to illustrate such changes (Fig. 3). For this period there was a greater increase during the day in February than in any other month.

The main features about the temperature were therefore the uniformity of the mean monthly temperatures; the considerable variation from month to month in the mean daily maximum temperature; the marked diurnal range in temperature, which varied considerably with the period of the year, and the increase in temperature during the day, which was greater in some months than in others.

Humidity. The mean daily percentage humidity varied considerably (Fig. 1). The highest monthly value was 91 % in August 1937 and the lowest 49 % in August 1938. The mean monthly values for 1937–9 inclusive varied from 57 % in December to

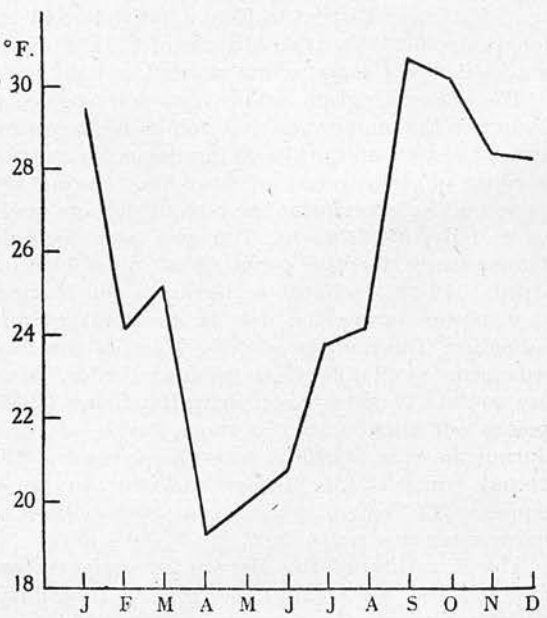

Fig. 2. Diurnal range in temperature.

78 % in May. The yearly figures for 1937, 1938 and 1939 were 73·5, 59·0 and 69·5 % respectively.

The wet- and dry-bulb temperatures during the period of the experiment were taken only once daily, at 8.30 a.m., and there are no data for humidity for this period, except for this time of

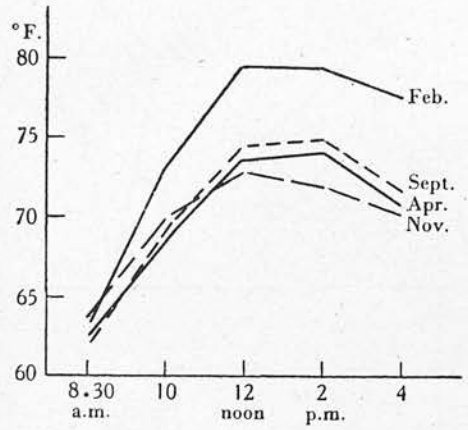

Fig. 3. Rise in temperature during day.

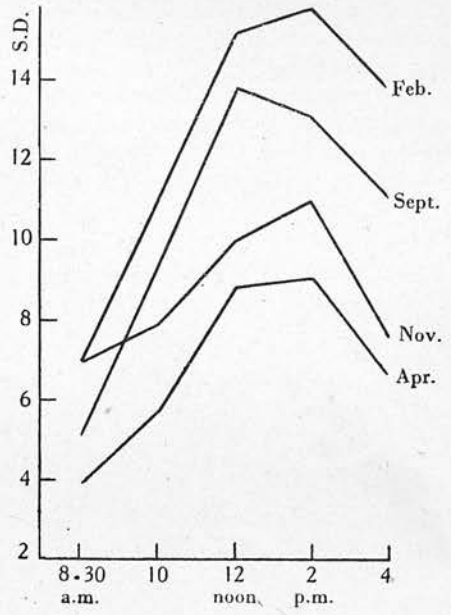

Fig. 4. Increase in saturation deficiency during day.

day. These temperatures were, however, taken at 8.30 a.m., 10 a.m., 12 noon, 2 p.m., and 4 p.m. from September 1941 to April 1942. The increase in the saturation deficiency during the day for different months in this period is shown in Fig. 4.

The main features about the humidity were therefore the marked diurnal and monthly variations.

Sunlight. The Experimental Station is practically on the equator, and there is therefore no appreciable change in the number of hours of daylight from month to month. There was, however, a distinct seasonal variation in the number of daily hours of sunshine (Fig. 5).

General. The data for saturation deficiency, maximum temperature, rainfall and daily hours of sunshine are summarized (smoothed) in Fig. 6, and climatographs based on the mean monthly values for the dry- and wet-bulb temperatures are shown in Fig. 7. The seaonal changes were quite clearly defined. In general the period of most rain was related to the period during which the saturation deficiency and the maximum temperature were lowest, and vice versa. The rainfall peak, however, occurred in April, and the lowest maximum temperature and saturation deficiency did not occur until May. The curves for maximum temperature and saturation deficiency follow each other fairly

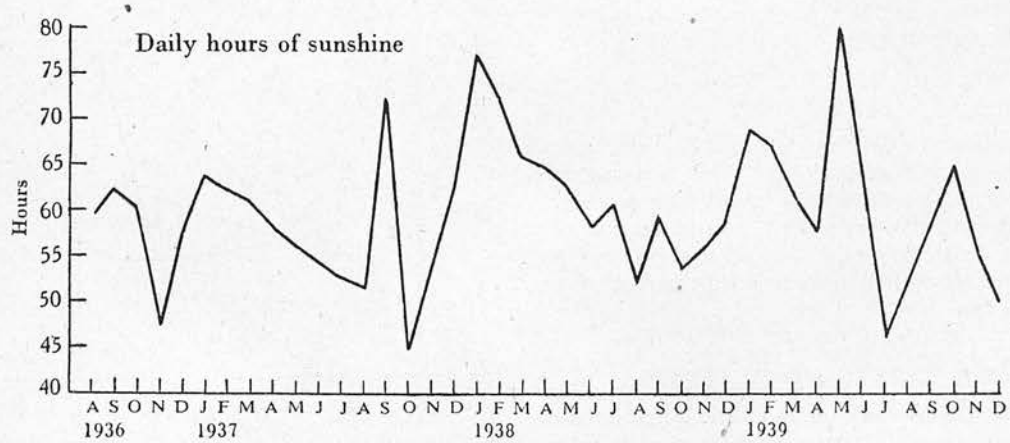

Fig. 5. Average number of daily hours of sunshine.

closely, the main difference being a sharp low point for temperature in May, while the saturation deficiency maintained a low level from May to August. The seasonal trend in the amount of sunshine is not unlike that for maximum temperature and saturation deficiency with the difference that (1) the low point for sunshine was in July, 2 months

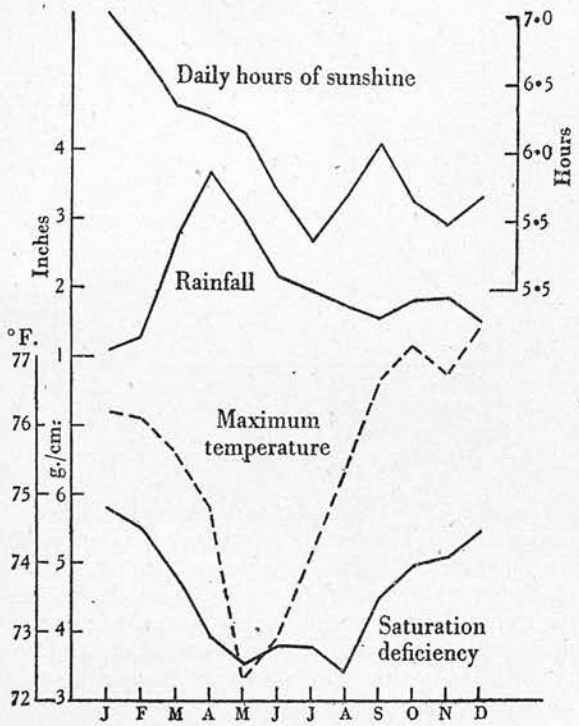

Fig. 6. Summary of meteorological data.

later than the low points for temperature, and (2) the hours of sunshine increased from July to a peak in September, while the maximum temperature increased from May to October. From October to December the curves for sunshine and temperature followed similar trends.

PASTURE

The pasture on the Experimental Station, apart from deficiency in sodium and chloride, is as rich as good British cultivated pasture (Orr & Holm, 1931). During the period of the investigation the animals subsisted solely on the pasture and received no supplementary feeding except in the case of the feeding experiment.

MATERIAL AND METHODS

The observations were made at the Experimental Station, Naivasha, Kenya, during the inclusive period from January 1936 to December 1939 on zebu and grade cows and heifers. The exact breeding of the grade cows was unknown, but they were

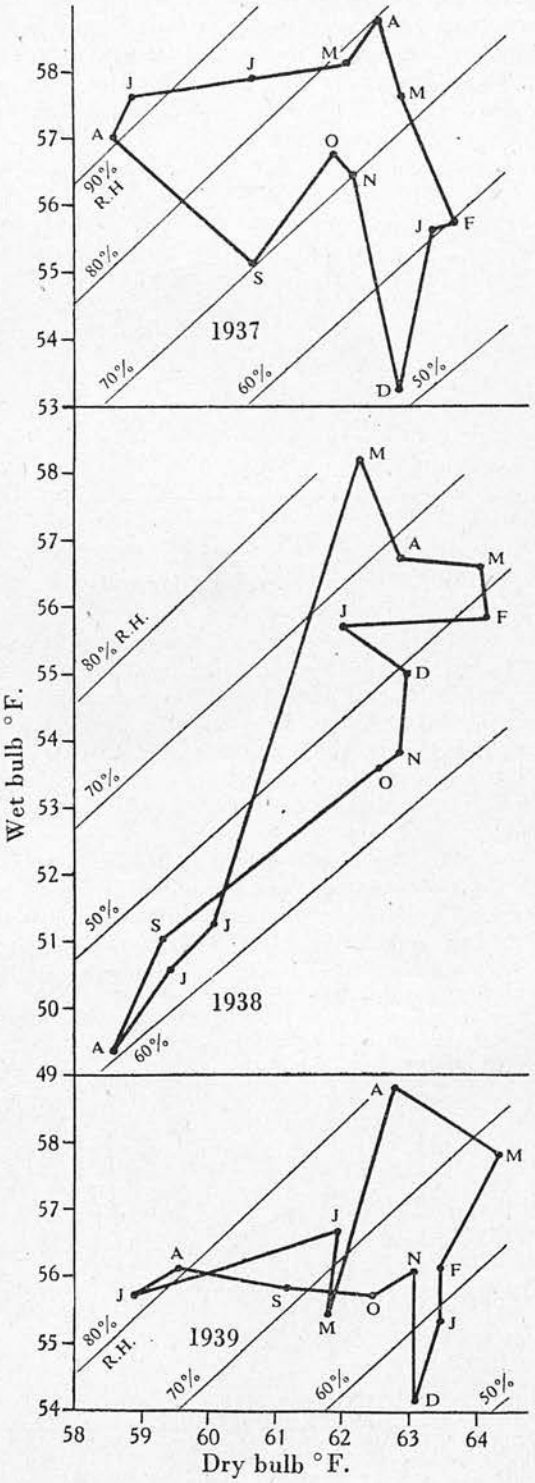

Fig. 7. Climatographs for 1937, 1938 and 1939.

of a high grade resulting from successive crosses of European bulls, mainly Shorthorn and Ayrshire, on zebu and grade cows.

The procedure adopted was essentially the same as that of the previous investigations (Anderson, 1936). Vasectomized bulls, of which there was an adequate number, were used to detect oestrus. One or two vasectomized bulls were always with the experimental animals. Cows which were about to come on heat (from about the 18th day after the last heat period) were placed in a small paddock at night where they were tested at 3-hourly intervals from 6 p.m. to 6 a.m. from 1936 to 1938 inclusive; in 1939, the test at 3 a.m. was omitted. The method adopted was therefore such that during the day the exact time of oestrus was known, and during the night the time was known within 3 hr., except in 1939, when there was an interval of 6 hr. from midnight to 6 a.m. when the animals were not tested. It will be seen from Table 5 that only a very small number of oestrous periods began between midnight and 6 a.m. Oestrous periods of which the exact time of onset of oestrus was unknown are not included in the data. During heat zebu animals were tested at 1-hourly intervals, and grade cows at 2 hr. after the onset of oestrus, and then at 1-hourly intervals. The duration of heat was therefore known to an accuracy of 1 hr.

The feeding experiment was continued from June 1936 to July 1937. The experimental animals were fed ad lib. a concentrate ration composed of maize 2 parts, bran 2 parts, blood meal ½ part, and hay. They were dosed daily, except on Sundays, with 1 oz. of the following mixture: salt 2 parts, lime 1 part, and bone meal 1 part. They were also dosed twice weekly with 1 oz. cod-liver oil.

For the light experiment, which lasted from July to November inclusive 1937, the experimental animals were housed at night in a small building, the walls of which were whitewashed. The source of light was a Tilley storm lantern. The light was turned on from dusk, about 6.30 p.m., to midnight, every night during the period.

The periodicity of oestrus

Particulars of the length of the dioestrous cycle in zebu and grade animals are given in Table 1, and the frequency distribution is shown in Fig. 8.

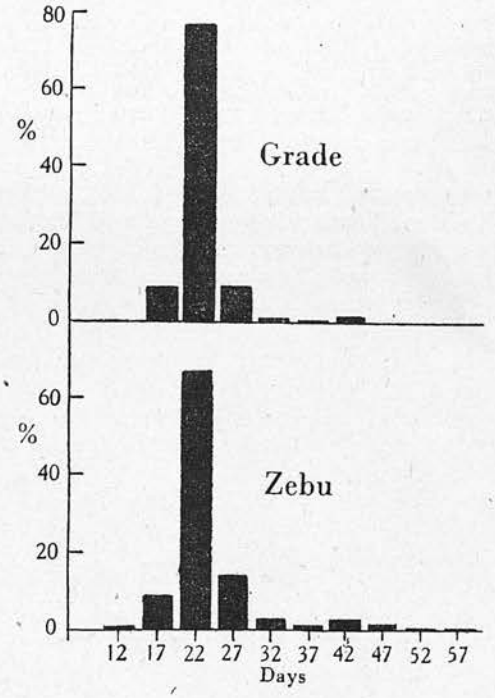

Fig. 8. Frequency distribution of length of cycle.

The cycle in the grade animals was slightly shorter than in the zebu animals ($t = 2{\cdot}103$, which just exceeds the 5 % level of 1·960).

Analysis of variance of cycle lengths for zebu animals showed a highly significant difference

Table 1. *Length of dioestrous cycle*

	1936	1937	1938	1939	Total
		Zebu			
No. of cycles	183	284	310	353	1130
Mean length in days	22·87 ± 0·42	22·28 ± 0·23	23·20 ± 0·36	23·52 ± 0·42	23·03 ± 0·19
Standard deviation	5·82	3·82	6·35	7·89	6·41
% cycles outside range 19–26 days	10·4	10·6	11·9	25·9	16·4
% of cycles below 19 days	1·1	1·8	1·6	8·5	3·7
% of cycles above 26 days	9·3	8·8	10·3	17·3	12·7
% of cycles in range 38–52 days	5·9	1·1	4·8	5·4	4·2
		Grade			
No. of cycles	68	174	225	209	676
Mean length in days	21·74 ± 0·63	21·34 ± 0·76	22·34 ± 0·37	23·73 ± 0·50	22·42 ± 0·22
Standard deviation	5·16	3·16	5·49	7·49	5·65
% cycles outside range 19–26 days	18·9	7·5	8·0	13·8	10·08
% of cycles below 19 days	8·1	2·9	2·2	1·4	2·35
% of cycles above 26 days	10·8	4·6	5·8	12·4	7·69
% of cycles in range 38–52 days	10·8	0·6	2·2	3·8	2·66

Table 2. *Monthly variation in length of dioestrous cycle*

Year	Jan.	Feb.	Mar.	Apl.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
						Zebu						
1936	22·0	24·2	21·3	23·7	21·9	24·6	21·1	22·8	21·9	22·1	27·8	22·3
1937	22·1	22·8	23·4	21·9	25·1	22·1	22·7	21·8	22·1	23·2	22·1	22·5
1938	22·7	22·2	22·8	21·3	25·2	23·8	22·6	23·4	22·8	22·7	23·8	21·9
1939	22·2	20·7	23·6	26·1	25·0	23·8	27·3	26·5	23·8	21·2	27·9	28·8
Mean	22·3	22·2	22·7	23·2	24·7	23·3	24·5	22·8	22·7	22·4	25·3	23·9
						Grade						
1936	—	—	—	—	—	—	—	—	20·3	21·3	21·5	22·5
1937	20·8	20·7	22·3	22·2	21·5	22·6	20·8	22·3	21·4	21·9	22·8	23·7
1938	21·2	24·3	22·6	23·5	22·1	25·4	23·1	21·9	21·8	24·2	23·4	21·5
1939	24·8	22·2	23·1	23·0	23·1	26·3	23·6	26·0	22·9	25·9	22·5	22·8
Mean	22·3	22·4	22·7	22·9	22·2	24·8	22·5	23·4	21·6	23·3	22·6	22·6

between cows and months for 1936, 1937, 1938 and 1939. In grade animals there was a highly significant difference between months in 1938 and a significant difference between cows in the same year.

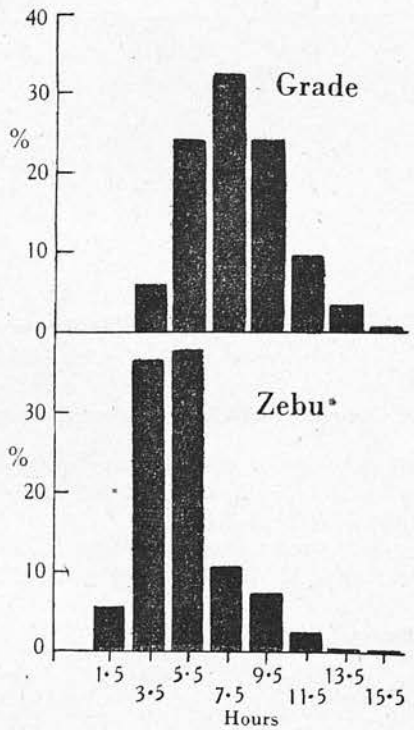

Fig. 9. Frequency distribution of duration of oestrus.

In the other years the differences between cows and months for grade animals was not significant. The difference in length of cycle was highly significant in different years between zebu and grade animals.

On the whole, the cycles of both zebu and grade animals showed marked regularity, particularly in the period 1936–8 inclusive, when cows were tested at 3-hourly intervals during the 24 hr. In 1939, when the 3 a.m. test was omitted, there was in both cases an increase in the percentage of cycles above 26 days in length. The coefficient of variation for zebu and grade animals was 27·8 and 25·2 % respectively. During 1936–8 the zebu animals had 25 cycles (i.e. 3·2 % of a total of 777 cycles) and the grades 8 cycles (i.e. 1·7 % of 467 cycles) of double length. During this period, therefore, 3·2 % of heat periods were probably missed in the zebu animals and 1·7 % in the grades. In these cases, heat either lasted less than 3 hr. or failed to occur. In 1939 the percentage of heat periods missed in zebu and grade animals was 5·7 and 4·7 % respectively. The difference between the zebu and grade cows is associated with the shorter heat periods in the zebu cows.

The zebu cycle (Fig. 10) was 23 days and over in length in May, June, October, November and December 1936; in April and May 1937, in May-August and October-November 1938; in April and May 1939, with a high level from July to December with peaks in July and November-December. The composite graph (Fig. 12) shows that the zebu cycle was over 23 days in length in April-August and October-December; the grade cycle was over 23 days in May-July, and there was a slight peak at 22·8 days in November (Fig. 12). The curves for monthly cycle lengths show very similar trends for both types of animals (Fig. 12).

In addition to the zebu cows which experienced heat regularly, there were four other zebu cows, not included on the above data, which behaved abnormally. These cows were under observation for 4 years from 1936 to 1939 inclusive. No. 8310 had 7 cycles in 1936, the average length of which was 21·7 days, and was not on heat again from June 1936 to December 1939. No. 8291 had 3 cycles in 1936 (34·4, 41·9 and 21·8 days in length), and also was not on heat in 1937, 1938 and 1939. Two others, nos. 8294 and 8302, failed to come on heat at all during 1937, 1938 and 1939. The ovaries of these cows during the periods of absence of heat were small and smooth with no palpable corpus luteum or large follicle, and they failed to undergo cyclical

Table 3. *Duration of oestrus*

	1936	1937	1938	1939	Total
		Zebu			
No. of periods	147	287	295	369	1098
Mean length in hours	3·67 ±0·22	5·90 ±0·15	4·27 ±0·06	4·74 ±0·08	4·78 ±0·07
Standard deviation	2·63	2·49	1·07	1·63	2·20
		Grade			
No. of periods	69	174	240	214	697
Mean length in hours	6·00 ±0·33	9·41 ±0·17	6·37 ±0·12	7·33 ±0·10	7·40 ±0·09
Standard deviation	2·78	2·22	1·79	1·60	2·38

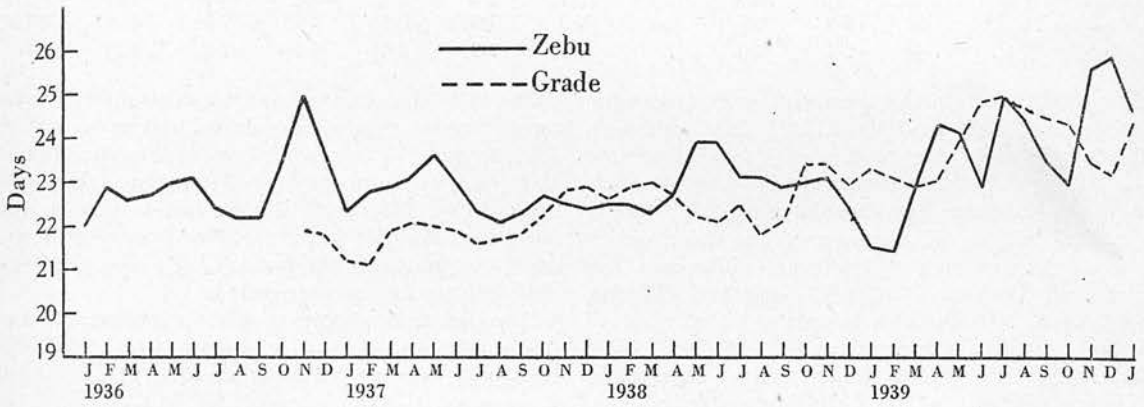

Fig. 10. Monthly variation in length of cycle.

changes. These four cows were also under observation in 1940 and 1941. No. 8291 came on heat irregularly. No. 8302 was on heat once in May 1940, and nos. 8294 and 8310 failed to come on heat at all. The two latter cows thus failed to come on heat at all during a period of 6 years. No. 8291, however, conceived at the end of 1941, showing that her cyclical irregularity was not incompatible with fertility.

The duration of oestrus

The duration of oestrus was significantly lower in grade than in zebu anaimals ($t = 7{\cdot}7$, which exceeds the 1 % level of significance). The coeffi-

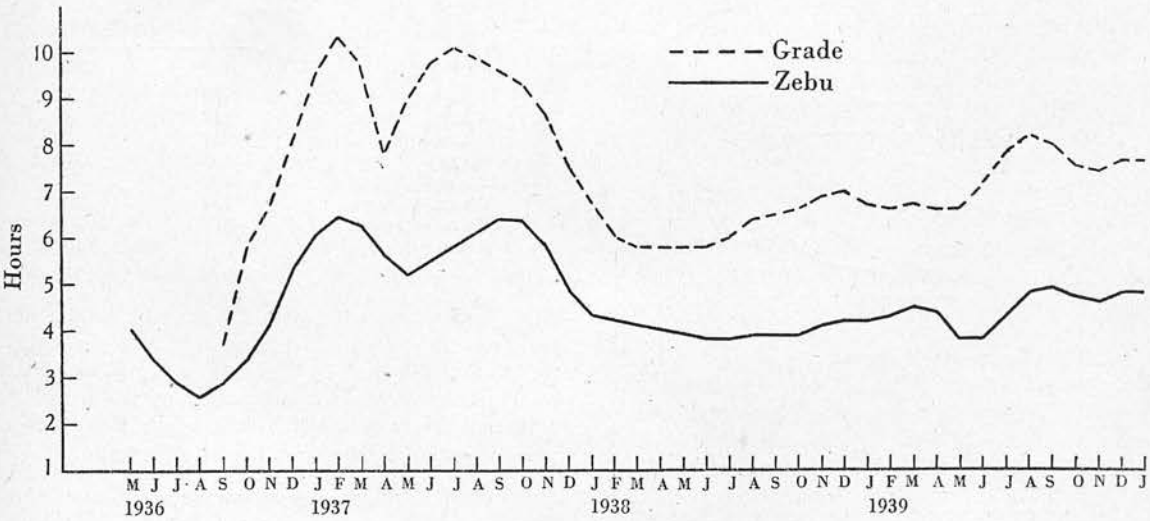

Fig. 11. Monthly variation in duration of oestrus.

Table 4. *Monthly variation in duration of oestrus*

Year	Jan.	Feb.	Mar.	Apl.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
						Zebu						
1936	—	—	—	—	4·1	3·3	3·0	2·4	2·7	3·6	3·6	6·0
1937	5·9	6·7	6·5	5·6	4·8	5·6	5·8	6·9	6·6	6·3	6·3	4·1
1938	4·6	4·0	4·2	3·8	4·0	3·7	3·7	4·0	3·9	3·6	4·5	4·5
1939	4·4	4·1	4·7	4·6	3·5	3·6	4·4	4·8	5·0	4·7	4·5	4·6
Mean	5·0	4·9	5·1	4·7	4·1	4·1	4·2	4·3	4·6	4·6	4·7	4·7
						Grade						
1936	—	—	—	—	—	—	—	—	3·7	6·6	6·2	7·9
1937	9·8	10·8	10·0	8·2	9·0	9·9	10·2	10·0	9·3	9·6	8·8	7·1
1938	6·9	5·7	5·8	5·7	5·8	5·7	5·8	6·8	6·2	6·7	6·9	7·0
1939	6·9	6·2	7·0	6·7	6·1	7·5	7·3	8·9	7·7	7·5	7·3	7·6
Mean	7·9	7·6	7·6	6·9	7·0	7·7	7·8	8·6	6·7	7·6	7·3	7·4

cient of variation in the length of oestrus for zebu and grade animals was 45·8 and 32·1 % respectively. The longest period recorded in each type of animal was 16 hr., and the shortest was less than 1 hr., but these two extremes were few in number (Table 3, Fig. 9). Analysis of variance of the duration of oestrus showed highly significant differences for cows and months in 1936, 1937, 1938 and 1939 for zebu animals. In grades there were highly significant differences between cows in 1936, 1937, 1938 and 1939. In 1936–7 there was a significant difference for months. In both types of animal the duration of oestrus varied in different years. It increased from 1936 to 1937, in which year it was longest, decreased in 1938 and increased again in 1939.

Monthly variations in the duration of oestrus are shown in Table 4 and Figs. 11 and 12 (smoothed data). The duration of oestrus showed the same trends in both zebu and grade animals in each year. In 1938 and 1939 the trends were fairly similar but they differed from those in 1937. The composite graph (Fig. 12) shows similar trends throughout the year. For the period October-December, however, the grade oestrus diminished, while there was little change in the zebu oestrus.

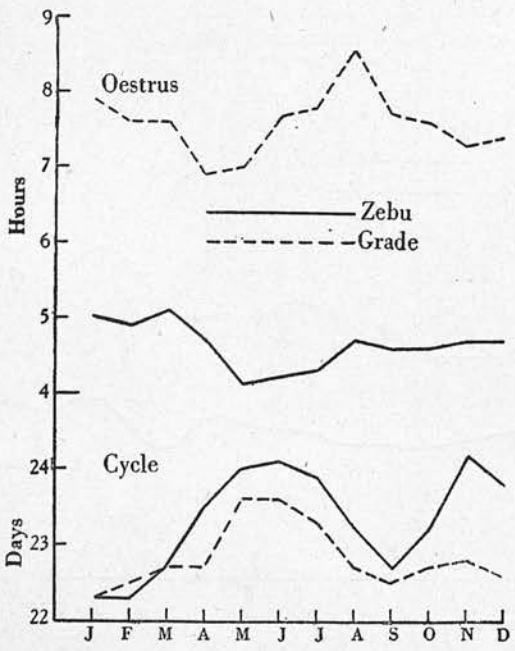

Fig. 12. Summary of oestrous cycle data.

The duration of oestrus, when experienced, was quite normal in the two zebu cows which came on heat only very occasionally in 1936. In no. 8310 it averaged 4 hr. and in no. 8291 3 hr.

Period of day and time at which oestrus started and ended

There were marked differences in the period of the 24 hr. at which oestrus usually began and ended. Zebu and grade cows were alike in this respect, so the data for both types have been grouped (Table 5). The majority of cows started

Table 5. *Period of day at which oestrus started and ended*

	No. of periods	
	Start	End
12 midnight–3 a.m.	3	133
3 a.m.– 6 a.m.	142	33
6 a.m.– 9 a.m.	722	49
9 a.m.–12 noon	162	378
12 noon– 3 p.m.	132	331
3 p.m.– 6 p.m.	434	282
6 p.m.– 9 p.m.	311	303
9 p.m.–12 p.m.	7	404
	1913	1913

their heat periods between 6 a.m. and 9 a.m., and the next most frequent period was between 3 p.m. and 6 p.m. followed by 6 p.m. to 9 p.m. The period at which heat ended is influenced by the length of heat and the time at which it started. Fewest heat periods ended between midnight and 9 a.m.

The pro-oestrous stage

By the pro-oestrous stage is meant the psychological state of the cow when she is coming on heat

and is followed by the bull, but will not stand for service. It lasts from the time the cow is first followed until the first service. This period is fairly constant in length. In zebu cows it lasted for 44–63 min. with an average of 55, and in grade for 42–87 min. with an average of 55 min. The coming on heat stage was thus fairly brief in both types of animal.

The effect of additional light

The exposure of zebu cows to artificial light for 5½–6 hr. after dark had no effect on either the duration of oestrus or on the length of the cycle (Table 6).

Table 6. *Effect of additional light*

	Experimental cows	Control cows
No. of cycles	33	85
Mean length in days	22·07 ±0·42	22·09 ±0·31
Standard deviation	2·37	2·84
No. of oestrous periods	34	93
Mean length in hours	5·76 ±0·32	6·39 ±0·24
Standard deviation	1·80	2·29

The effect of a supplementary ration

Feeding a supplementary ration had no effect on either the duration of oestrus, or on the length of the cycle, which indicates that during the period of the experiment the nutritive value of the grazing was adequate for the normal expression of the oestrus cycle (Table 7).

Table 7. *Effect of supplementary ration*

	Experimental cows	Control cows
No. of cycles	74	180
Mean length in days	22·46 ±0·43	22·57 ±0·38
Standard deviation	3·77	5·41
No. of oestrous periods	87	191
Mean length in hours	4·60 ±0·29	4·67 ±0·22
Standard deviation	2·50	3·04

Discussion

The mean length of the cycle (23·03 days) in the zebu animals at Naivasha was greater than that previously recorded for zebus at Sangalo (20·1 days; Anderson, 1936). This difference may possibly have been related to individuality and/or age changes, for the Naivasha animals were, on the whole, considerably older than the Sangalo ones. Hammond (1927) has observed that cows have longer cycles than heifers and there is, on the whole, an upward trend in cycle length in both zebus and grades discernible in Fig. 10, which may be associated with age. The grade cycle (22·42 days) was longer than the average usually recorded for European cows (19–22 days) in their own environment, but a considerable range of variation has been recorded for the latter. On the whole, however, it appears that cycle length is somewhat greater in Kenya than in temperate countries.

The zebu cycle showed greater seasonal variation than the grade cycle. From November 1936 to the beginning of 1938 the trends in both types of animal were fairly similar, but thereafter there were more discrepancies due to the greater variation in zebu cycle. The relation between sexual activity and meteorological conditions is discussed later. Here, it may be stated that it is believed that such a relationship does exist. Meteorological conditions vary considerably from month to month and from season to season, and so must any effect they may have on animals. In the combined graph (Fig. 12) of the whole experimental period, more uniform seasonal trends are obvious, with the grade cycle at a lower level throughout.

In respect of duration of oestrus, grade animals are intermediate between zebus in Kenya, and European animals in temperate countries. It has not been possible to make observations on pure-bred animals of European breeds in Kenya, and it is impossible to say what would be the effect of temperate conditions on zebus and grades. The zebu animals did not react to additional light, but whether this was due to their insusceptibility to light or to the inadequacy of the light is not clear. It would seem, however, that the longer oestrus in grades may be due to genetic factors. Although, on the one hand, it is conceivable that zebu animals and animals with a high proportion of European blood have become conditioned in the course of time to different climatic conditions, on the other hand, both types of animals may react somewhat similarly to the same climatic conditions, as the evidence presented later in this paper indicates.

The seasonal trends in the duration of oestrus are remarkably similar throughout the whole period from 1936 to 1939. Considering the data as a whole (Fig. 12) the periods with longer cycles are approximately those with shorter heat periods and vice versa. This relationship is particularly noticeable when comparing the zebu cycle with the grade oestrus. Shorter cycles and longer heat periods may be regarded as an indication of increased sexual function and longer cycles and shorter oestrus as depressed sexual function.

CLIMATIC CONDITIONS AND SEXUAL ACTIVITY

For the examination of the relationship between climatic conditions and sexual function, it appears best to consider, in the first place, the average data shown in Figs. 6 and 12 and to observe any general correlation or tendency, and then both sets of data for the different years.

The increase in cycle length from January to May-June was associated in time with a decrease in the maximum temperature; the decrease in the cycle from June to September was associated with an increase in temperature which continued to October; the increase in the cycle from October to November was associated with a decrease in temperature, while a decrease in the cycle from November to December was associated with an increase in temperature. Thus, except for the period September-October, there was a good agreement between cycle length and maximum temperature, in that an increase in temperature was associated with a decrease in cycle length and vice versa. The seasonal variation in the duration of oestrus bears, as has been pointed out, a general relationship to that of the cycle.

Comparing sunshine with cycle length in the first 6 months of the year, the decrease in cycle length was associated with a decrease in the daily amount of sunshine, although the latter continued to July; the decrease in the cycle from July to September was associated with an increase in sunshine; the increase in the cycle from September to November was associated with a decrease in sunshine; and the decrease in cycle length from November to December was associated with an increase in sunshine. Thus, except for the month of July, there was a good agreement between sunshine and cycle length in that a decrease in cycle length was associated with an increase in sunshine and vice versa.

The general conclusion which it seems permissible to draw from these average data (Figs. 6, 12) is that a period of increased sunshine and temperature is related to increased sexual activity, and a period of decreased temperature and sunshine is related to decreased sexual activity. Also, as has been pointed out (under physiographical conditions) in general, the season of most rain is related to the period during which the saturation deficiency and maximum temperature are lowest and vice versa, although there is not an exact coincidence in time.

When the data for zebu cycle length in each year (Fig. 10) is compared with temperature (Fig. 1) and sunshine (Fig. 5) the correlation is much less obvious, though, on the whole, there is, in general, more agreement than disagreement. Comparing the months from November 1936, for which an increase in zebu cycle length was associated with decrease in temperature or amount of sunshine and vice versa, there was 68 % agreement for temperature and 82 % agreement for sunshine; for the grade cycle the figures were only 49 % for temperature and 46 % for sunshine. Comparing the months for which an increase in zebu oestrus was associated with an increase in temperature or sunshine and vice versa, there was 70 % agreement with temperature and 57 % with sunshine; for grade oestrus the figures were 51·3 % for temperature and 54 % for sunshine. On this method of computation it was only the zebu animals that showed marked relationship between sexual activity and temperature and sunshine. Further, the grade animals had a statistically significant seasonal variation in the cycle only in 1938, and in oestrus only in 1936–7. Changes in meteorological conditions are much less in some years than in others (e.g. in 1938, temperature changes were more marked than in other years), and any effect they may have on sexual function will vary according to the degree and extent of the changes. Also, there may be a time interval before the animal responds to any external factors.

DISCUSSION

In a previous investigation of the possible influence of climatic and nutritional factors on the periodicity and duration of oestrus in zebu cattle, made in 1933–4 at Sangalo, Kenya, no relationship was found between sexual activity, rainfall and pasture constituents. It was thought at that time that there might have been a possible relationship between sunshine and sexual activity, but no method was then available for measuring the actual duration of sunshine.

The restricted nutritional regime the zebu cows experienced at Sangalo probably prevented the full expression of oestrus and would therefore obscure to some extent the seasonal variation in oestrus. Oestrus did, however, occur with cyclical regularity, and the seasonal variation showed two main peaks at approximately the same period of the year as at Naivasha, although the rainfall differed in amount and distribution at both places. As at Naivasha the long rains were followed by longer cycles, but the short rainfall peak in August was associated with a decrease in cycle length. At Naivasha the increase in rainfall and the probable associated pasture changes were related in time to depressed sexual activity. At Naivasha nutritional conditions were adequate; at Sangalo the pasture was relatively deficient in protein and phosphorus. Similar seasonal variations in cycle length have thus been obtained under different conditions of rainfall and nutrition, and therefore, within the limits of these observations, it would appear that neither of these factors were involved.

There are two possible ways in which temperature and sunshine may affect sexual functions in cows: (*a*) by altering the metabolism generally, and (*b*) by a more specific effect of light on sexual function. With regard to the former view, Ritzmann & Benedict (1938) noted that during the

summer months the basal metabolism of dairy and beef cattle was significantly increased over the autumn and winter months, and they associated this with high temperatures and increased solar radiation during the summer months. The longer periods of oestrus experienced by cows in Britain in the summer (Hammond, 1927) may be related to this fact. There is little experimental evidence relating metabolism to sexual function, but it seems that any condition which elevates or depresses the metabolism of the body as a whole must also have a corresponding effect on the reproductive system. Clinically, thyroid therapy in women is often beneficial for reproductive disorders, particularly when associated with a low basal metabolic rate. In the rat, exposure to cold lengthens the oestrous cycle, and it is thought that this change is brought about through the lowered general metabolic level, since body temperature and general activity are reduced (Lee, 1926).

Of all the climatic factors investigated, light is the one which has been found by experiment and observation to affect reproduction in birds, mice and ferrets (Marshall, 1936; Bissonnette, 1936). The data on this subject were discussed briefly in the previous communication on the influence of environmental factors on reproduction in cattle (Anderson, 1936). In the present investigation the number of daily hours of sunshine appears to be more closely associated with reproductive activity than any of the other climatic factors. One point against a specific effect of light was the failure to influence reproductive activity in zebus exposed to additional light, but this question must remain open until the effect of light of different intensities and wave-lengths has been tested.

The animal's reaction to climatic factors will condition to a large extent any seasonal variation in sexual activity. Zebus are presumably better adapted to the tropical plateau climatic conditions in Kenya than are grades, and it might therefore be expected that they would react more to such seasonal climatic changes as do occur, which is indeed the case for sexual function, under tropical conditions. High-bred cattle, though bred in Kenya, have inherent characteristics adapted to a temperate climate and may show a somewhat different response to climatic conditions compared with zebu cattle. On the whole, however, the general similarity of the seasonal variation in sexual activity (in both types of cattle), though of much lesser degree in grades, seems to indicate that environmental conditions are primarily responsible.

The heat-regulatory mechanism is more severely tested in cattle of European types than in zebus. The effect of temperature on cattle has been investigated by several workers lately, notably by Rhoad (1936, 1940), who found that external temperatures above 73° F. greatly increased the metabolism of European cattle and only slightly that of zebu cattle. Regan & Richardson (1938) found that temperatures above 70° F. reduced milk production and altered its composition in European breeds, and temperatures of 80–85° F. had very marked effects. Hays (1926) also gives 70° F. as the temperature above which the increased metabolism was sufficient to influence mammary secretion.

Observations in Kenya, including Naivasha, have shown that under natural grazing conditions, the heat-regulatory mechanism of grade cattle allows the rectal temperature to rise above 102° F. once a shade temperature of 70° F. is passed (Daubney, 1942). Rhoad (1936) has pointed out that the degree to which the loss of energy as the result of high environmental temperature is detrimental, is in direct relation to the time period the cattle are subjected to tropical conditions. While short periods of extreme thermal conditions may be borne without endangering heat or body functions, prolonged periods of the same abnormal conditions may severely affect health and production. It has been noted in Kenya (unpublished data) that the body temperature of grade cattle exposed to high temperatures may continue to rise after the body temperature of zebu cattle, exposed to exactly the same conditions, has begun to fall. Also, since the body temperature of grades rises higher than that of zebu animals, it takes longer to return to normal. Thus, apart from the actual temperature to which the animal is exposed, the period of exposure has a greater effect in grades. Under conditions of high environmental temperature, the stimulation to metabolism is presumably greater in grades than in zebus, but the greater loss of energy in grades may have a depressing effect on sexual function.

This investigation has shown a relationship, in time at least, between sexual function and climatic conditions. A season of increased sexual activity is associated with an increase in temperature and sunshine and decrease in humidity, and vice versa. Whether the effect is a complex one, involving these and perhaps additional factors, or whether it is restricted to any one of them, cannot be decided at present, but it appears possible that the effect may be exerted through the metabolism. It has been shown by Ritzmann & Benedict (1938) that previous nutritional treatment affects the basal metabolism, so any effect of environmental conditions is not likely to be a simple one.

SUMMARY

Records are given for 1130 oestrous cycles in zebus and 678 cycles in grades. The mean length was 23·03 days in zebus and 22·42 days in grades. The mean duration of oestrus was 4·78 hr. in zebus.

7·40 hr. in grades. Neither exposure to additional light at night nor feeding a supplementary ration had any effect on the cycle of oestrus.

Seasonal variations in both the cycle and oestrus occurred in zebus, but in grades they were much less marked. An association between climatic conditions and sexual function was noted in that a season of increased temperature and sunshine was associated with increased sexual function and vice versa.

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THE CHANGE IN THE *p*H OF THE SEMEN OF THE BULL AFTER INCUBATION

By JAMES ANDERSON, *Experimental Station, Naivasha, Kenya*

It was shown recently that the initial *p*H of the semen of the bull and the degree and direction of the change in *p*H during storage were of primary importance for the retention of high motility in stored semen (Anderson, 1942). Motility was best retained when the *p*H decreased steadily throughout the period of storage. It was therefore decided to investigate the alteration in the *p*H of semen after 1 hr. incubation at 37° C. to see if the *p*H change in this short period had any relation to the motility during storage; and also to study the relationship of the *p*H change to other characteristics of semen, such as the initial *p*H, the motility, and the number of spermatozoa per c.mm., with the object of determining, if possible, a simple, accurate and comprehensive method of evaluation of the semen of the bull at the time of collection. The results of this experiment show that a significant relationship does exist between the *p*H change after 1 hr. incubation and the other characteristics, and this estimation of the *p*H change may prove of practical value as it probably provides a measurement of the functional activity of the spermatozoa.

MATERIAL AND METHODS

The semen was collected with the artificial vagina in the usual way. For incubation, 0·5 ml. of semen was placed in a small pyrex test-tube open to the air, and kept for 1 hr. in a Thermos flask at 37° C. The *p*H was determined with the Beckman glass electrode before and after incubation at a temperature of 33° C. Counts of the spermatozoa were made with the Buerker haemocytometer and the motility was expressed as the percentage of spermatozoa showing very active movement. The semen was stored as previously indicated (Anderson, 1942) either undiluted or diluted with egg yolk-phosphate medium (Phillips, 1939), and the temperature was reduced gradually in the following manner: immediately after collection the semen was held at a temperature of 30° C. for 2–3 min., during the *p*H and motility estimations; it was then transferred to 25° C. for 15 min., followed by 15 min. at 20° C. and 1 hr. at 15° C. and stored at 8–10° C.

RESULTS

The mean change in the *p*H of 134 ejaculates after incubation for 1 hr. at 37° C. was $-0{\cdot}302 \pm 0{\cdot}027$ ($s = 0{\cdot}318$). Analysis of variance (Snedecor, 1938) of the change in *p*H for seven bulls on the Experimental Station for a period of 4 months from December 1941 to March 1942 (Table 1) showed highly significant differences between bulls. The range in the *p*H change in the ejaculates of these bulls was from $-0{\cdot}87$ to $+0{\cdot}36$, and the average values for individual bulls varied from $-0{\cdot}460$ to $+0{\cdot}113$. The monthly values for the *p*H change were not statistically significant over the short period of the experiment.

Table 1. *Analysis of variance in change in p*H

Source of variance	D.F.	Sums of squares	Mean square
Bull	6	3·7130	0·6188
Month	3	0·3927	0·1309
Bull-month interaction	18	1·5125	0·0840
Within subclasses	81	4·4425	0·0548
	108	10·0607	

A highly significant ~~negative~~ positive correlation was noted between the change in *p*H, the number of spermatozoa per c.mm. and the initial motility, in that the greater the decrease in *p*H on incubation, the higher the degree of initial motility, and the greater the concentration of spermatozoa per c.mm. There was a highly significant ~~positive~~ negative correlation between the change in *p*H and the initial *p*H in that the greater the *p*H decrease the lower was the initial *p*H (Table 2).

Although these relationships did occur, there were some exceptions. Six ejaculates with 1,250,000 or more spermatozoa per c.mm. had a *p*H change of less than $-0{\cdot}29$; the average change for these six ejaculates was $-0{\cdot}05$ and three of the ejaculates had plus values. In contrast, two ejaculates with

Table 2. *Correlation and regression of p*H *change*

	No.	Mean	*s*	*r*	Regression coefficient	S.E. o.e.	*t*
*p*H change and concentration	134	$-0{\cdot}302 \pm 0{\cdot}027$	0·318	$+0{\cdot}3751$	$E = 0{\cdot}123 \pm 0{\cdot}00068$	0·273	5·06
*p*H change and % motility	134	$69 \pm 2{\cdot}4$	27·7	$+0{\cdot}6205$	$E = 0{\cdot}186 \pm 0{\cdot}0071X$	0·249	9·10
*p*H change and initial *p*H	134	$6{\cdot}73 \pm 0{\cdot}017$	0·198	$-0{\cdot}3163$	$E = 0{\cdot}508X \pm 3{\cdot}721$	0·286	9·25

The *p*H values are the same for the three groups of data.

Concen ion(000's) Mean 665.2 ± 12.1 S 444.1

less than 250,000 spermatozoa per c.mm. had a *p*H change value −0·30 or more.

Eight ejaculates had an initial *p*H of 7·11 or higher, and these ejaculates showed either poor or no activity on collection and spermatozoa were few or absent. Seven of these ejaculates had plus *p*H change values and one ejaculate had a *p*H value of −0·06. Four ejaculates with an initial *p*H of 6·50 and under had *p*H changes of less than −0·29.

There was a highly significant difference ($t = 10{\cdot}37$) when the *p*H change was compared for ejaculates with an initial motility of over or under 70 % (Table 3). Taking an initial *p*H of 7·10 and under, together with the degree of *p*H change in comparison with initial motility, there were (1) six ejaculates (4·5 %) with a *p*H change of −0·30 or more (average −0·37) associated with a motility of 60 % or less, and (2) conversely, there were seventeen ejaculates (12·7 %) with a *p*H change of −0·29 or less (average −0·112, range −0·29 to +0·23) and a motility of over 70 % (average 81 %). The six ejaculates under (1) had an average initial *p*H of 6·77 (range 6·39–7·01) and an average number of spermatozoa per c.mm. of 610,000 (range 360,000–1,180,000). The seventeen ejaculates under (2) had an initial *p*H of 6·72 (range 6·38–7·06) and an average number of spermatozoa per c.mm. of 694,700 (range 160,000–1,590,000). On a basis of *p*H 7·10 and under on collection and a *p*H change of −0·30 or more after incubation for 1 hr. at 37° C. the six ejaculates under (1) would be classed as good and the seventeen ejaculates under (2) as bad. Therefore, on this classification twenty-three ejaculates (17·2 %) did not agree with the motility estimations.

Table 3. *Change in pH and initial motility*

	No.	*p*H change mean	*s*
(1) Motility 70 % and over	95	−0·437±0·0221	0·219
(2) Under 70 % motility	39	−0·013±0·034	0·212
	$t = 10{\cdot}37$		

There were eighty-one ejaculates of 80–100 % motility on collection for which the relationship between the *p*H change and motility after incubation could be examined. The motility at 1 hr. was expressed as the percentage decrease of the initial motility, all of which was regarded as 100 % for this purpose. The change in *p*H was associated with a decrease in the motility of undiluted semen (Table 4; $r = 0{\cdot}3034$, which is a highly significant difference). Therefore, the greater the *p*H change, the poorer was the motility after incubation. A comparison between the characteristics of undiluted semen of 80–100 % motility on collection which showed a decrease in motility of (1) 40 % or less and (2) 50 % or more, is shown in Table 5. There were highly significant differences in the three pairs of data (for the initial *p*H, $t = 7{\cdot}07$; for *p*H change $t = 3{\cdot}01$; for number of spermatozoa per c.mm. $t = 4{\cdot}28$). The lower the initial *p*H the greater the decrease in *p*H on incubation, and the greater the number of spermatozoa per c.mm. the greater was the decrease in motility after incubation for 1 hr.

Table 4. *Change in pH and percentage decrease in motility in undiluted semen*

	No.	Mean	*s*	*r*
*p*H change	81	−0·445±0·030	0·2115	0·3034
Motility	81	30·2 ±2·80	25·24	

There was, however, a smaller decrease in the motility of semen diluted 1:1 with egg-phosphate medium compared with undiluted semen (Table 6, $t = 3{\cdot}27$, which is a highly significant difference). The better motility of diluted semen was in agreement with the smaller *p*H change in this medium (Table 7).

Table 5. *Comparison between characteristics of undiluted semen kept for 1 hr. at 37° C. with a percentage decrease in motility of* (1) 40 % *and less, and* (2) 50 % *and more*

	Initial *p*H	
	Mean	*s*
(1) 40 % and less	6·696±0·021	0·131
(2) 50 % and more	6·472±0·018	0·135
	$t = 7{\cdot}07$	

	*p*H change	
	Mean	*s*
(1) 40 % and less	−0·432±0·025	0·158
(2) 50 % and more	−0·532±0·057	0·278
	$t = 3{\cdot}01$	

	Concentration	
	Mean	*s*
(1) 40 % and less	675·5±54·38	339·6
(2) 50 % and more	1070·5±75·48	364·8
	$t = 4{\cdot}28$	

The available data were examined for evidence of a correlation during storage between the period of time for which a high motility (70 % and over) was maintained and (1) the *p*H change after incubation for 1 hr. at 37° C (eighty-four ejaculates), (2) the decrease in motility in undiluted semen (sixty-two ejaculates), and (3) the decrease in motility in semen diluted 1:1 with egg-phosphate medium (fifty-six ejaculates) all with completely negative results, the correlations being statistically insignificant in each case.

Table 6. *Relation between percentage decrease in motility in undiluted semen and semen diluted* 1:1 *with egg-phosphate medium*

	Mean	s
Undiluted semen	36·91 ±3·07	25·34
Diluted semen	24·40 ±2·27	18·75

It may conveniently be stated at this point that further data on the relation of low initial pH to the maintenance of high motility during storage have been obtained. In a series of eighty-four ejaculates diluted 1:1 with egg-phosphate medium, 70 % and over motility was retained by (1) forty-seven ejaculates with an initial pH of 6·41–6·70 for an average of 3 days, (2) twenty-five ejaculates with an initial pH of 6·71–6·90 for an average of 2·28 days, and (3) twelve ejaculates with an initial pH of 6·91–7·10 for an average of 1·67 days. Thus the initial pH of undiluted semen is associated with the motility on storage whether the semen is diluted or not.

Table 7. pH *change in semen* (1) *undiluted and* (2) *diluted* 1:1 *with egg-phosphate medium*

	No.	Mean	s
Undiluted semen	95	−0·360 ±0·030	0·291
Diluted semen	84	−0·197 ±0·022	0·169

$t = *0·5897$

DISCUSSION

The decrease in the pH of the semen of the bull after incubation is probably due to the metabolic activity of the spermatozoa. Smith & Asdell (1940) have studied the buffering capacity of bull semen and their examples show it to be relatively constant. It is conceivable that differences in the buffering capacity of semen will affect the pH change, but the impression from the present experiments is that the pH change is due, primarily at least, to the vital activity of the spermatozoa themselves, for the pH change is greater in 1 ml., compared with 0·5 ml. of the same undiluted ejaculate and pH changes occurred in the well-buffered egg-phosphate medium, though of a lesser degree, which were positively correlated with pH changes in undiluted semen. The smaller changes in the diluted semen were probably related to the reduction in the number of spermatozoa per c.mm. but may also have been partly due to the buffering capacity of the egg-phosphate medium.

With regard to the evaluation of semen at the time of collection the object is to determine to what extent the pH change agrees with or differs from other methods of estimation, such as the initial pH of semen, the degree of motility and the concentration of spermatozoa. There is clearly a need for a better estimation of semen than that provided by the latter characteristics. The ultimate object of evaluation of semen is the determination of fertilizing capacity, and our present knowledge leaves much to be desired in this respect. It is possible at present to identify a characteristic type of semen with fertility, sterility or with 'reduced' or 'doubtful' fertility (Anderson, 1941; see this paper for other references), but the significance of deviations in one or other respect of semen characteristics is largely unknown. So far the respiratory rate of spermatozoa is the measurement which gives the best indication of fertility (Edwards & Walton, 1939).

It must be stated at this point that the estimate of motility, although of considerable practical value, is largely subjective and is not therefore an exact one. With experience an observer will form a very good impression of the relative degree of motility in different ejaculates, but it is doubtful if workers in different countries will make exactly the same estimate of motility. There is therefore a need for an exact measurement to replace the estimation of motility. Chang & Walton (1940) have stated that there was good agreement between the respiratory rate of ram spermatozoa and motility. The present investigations show that there is a highly significant relationship between the pH change and motility. For practical purposes of artificial insemination of cattle, 70 % motility has been regarded as the arbitrary border-line and ejaculates of less than 70 % motility are not used for insemination, though it is quite possible that ejaculates of lesser motility would prove fertile. The lack of agreement in 17 % of ejaculates between pH change and motility may be due to the inexactness of the motility estimates, or it may be that the vitality of spermatozoa, as illustrated by the pH change, is not always consonant with motility. It is also clear that the vitality of spermatozoa is not always high when other semen characteristics are good, as, for example, a pH change of less than −0·29 with ejaculates of low initial pH, containing a large number of spermatozoa and conversely the vitality may be high when motility is poor and the number of spermatozoa is amall. The vitality, however, was not high when the initial pH was 7·11 or higher. In general, therefore, the pH change provides an exact measurement of semen, which agrees closely with the measurements of initial pH, the number of spermatozoa per c.mm. and the degree of motility.

It was shown previously, in confirmation of other work (Anderson, 1942), that there was a significant inverse relationship between the pH of semen of the bull, the concentration of spermatozoa, and the motility and the volume of the ejaculate; it was suggested that this negative correlation should prove useful for the evaluation of semen. Further

confirmation of the significance of the inverse relationship between the initial *p*H of the semen, the concentration of spermatozoa and the degree of motility are provided by the 134 ejaculates which form the basis of the experiments of *p*H change. It was therefore concluded that the determination of the *p*H would give an indication of the concentration as well as the motility of the spermatozoa, in that the lower the *p*H the greater was the number of spermatozoa per c.mm. and the higher the degree of motility. There were, however, about 15 % of ejaculates for which this negative correlation did not hold and for which the initial *p*H did not agree with the motility and concentration.

The *p*H change stands in a similar relationship to the motility and concentration of spermatozoa as the initial *p*H, for the *p*H change and the initial *p*H are ~~positively~~ correlated, ~~and both bear a negative correlation to the motility and concentration of spermatozoa~~. The *p*H change is therefore confirmatory of the initial *p*H. In this series of 134 ejaculates there were but few cases in which the initial *p*H did not agree with the *p*H change and these cases—four in number—had a low initial *p*H and a *p*H change of less than $-0{\cdot}29$. It is suggested that the determination of the initial *p*H and the *p*H change after 1 hr. incubation, representing, it may be, both the functional state of the male reproductive organs and the functional activity of the spermatozoa will provide an accurate evaluation of the semen of the bull.

There were considerable differences in the degree of activity after 1 hr. incubation between undiluted ejaculates, and these changes were associated with the vitality of the spermatozoa (Table 4). It would seem that seminal fluid is not on the whole a good medium for the maintenance of high motility, and the greater the metabolic activity of the spermatozoa the sooner the nutritive capacity of the semen was exhausted. Ejaculates differ considerably for example in their glucose content, and during storage glycolysis is more marked in some ejaculates than in others (unpublished data). In semen diluted with egg-phosphate medium and incubated for 1 hr., the resultant motility is considerably better than in the undiluted semen. Also, in storage experiments the motility of spermatozoa in undiluted ejaculates is much less well maintained, compared with, for example, semen diluted with egg phosphate. The more acid end-point in undiluted semen after incubation is probably not of importance, for there was considerable variation in the percentage decrease in motility with equally low *p*H end-points.

When bull semen was stored for a period of days in egg-phosphate medium there was no correlation between maintenance of high motility and the *p*H change in either the undiluted or the diluted fractions of the ejaculates. The ejaculates that were used for storage in this instance showed, however, negative *p*H changes. For the undiluted fractions the mean *p*H change was $-0{\cdot}439$ ($s = 0{\cdot}218$). This result would seem to indicate a deficiency in this medium or storage method, and support for this view is obtained from the abruptness with which the motility decreases in egg-phosphate diluted semen at the end of the storage period—the motility falls from 70 % and over to nil or poor motility in the course of 24 hr. The *p*H change may prove of more use when better storage methods are available which permit a longer life of spermatozoa *in vitro* and allow differences in their vitality to become apparent. At present the *p*H change in conjunction with the initial *p*H may be used to decide which ejaculates are fit for storage.

SUMMARY

The mean change in the *p*H of 134 undiluted ejaculates of clinically normal bulls was $-0{\cdot}302 \pm 0{\cdot}027$ after 1 hr. storage at 37° C. The greater the *p*H change, the lower was the initial *p*H and the higher was the initial motility and the number of spermatozoa per c.mm. The greater the decrease in *p*H the poorer was the motility after incubation. The motility was better maintained in semen diluted with egg-phosphate medium, compared with undiluted semen, which agreed with the smaller *p*H change in this medium. There was no evidence of a relationship between the *p*H change and the motility after incubation and the period of time for which a high motility was maintained during storage. It is suggested that the determination of the initial *p*H and the *p*H change after 1 hr. incubation may provide a useful and accurate evaluation of the semen of the bull.

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Price 7s. 6d.

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FOREWORD

INTEREST in artificial insemination has increased enormously in recent years. Its possibilities as a method to aid the development and improvement of livestock production, especially of dairy cattle, and in disease control are becoming widely realised. Its use as an experimental technique has been extended in many economic animals. A great deal of its practical application has been attempted without satisfactory knowledge of the underlying biological and physiological principles ; this has undoubtedly stimulated much of the study recently devoted to its scientific basis, but it would be incorrect to ascribe even the major contributions to the utilitarian aspects of the method.

In 1933 the Imperial Bureau of Animal Breeding and Genetics published a review of the subject prepared by Dr Arthur Walton ; other publications have since then appeared from various sources, and have not only served as technical handbooks but also added impetus to the demands for further information which all workers and scientific information services continually receive. In the present communication Dr James Anderson gives a comprehensive summary of the voluminous literature which has accumulated on the various aspects of the subject.

The publication is in three parts. Part I is directed particularly to the need of the scientific worker for a detailed review of the literature dealing with the study of semen, the factors which affect its production, and the properties and reactions which affect its use for insemination. Part II embraces a discussion of the use of artificial insemination in the different species, of its application in different countries and circumstances, and of the limitations imposed on its use by various factors, including those arising from the physiological processes of female reproduction. Part III describes the actual techniques of semen collection, examination and handling, and of insemination ; it is thus the section of most direct interest to those concerned with the practical application of the method.

The main text covers literature up to late 1943 ; additional matter and later information are given in a Supplement. The sectional treatment followed throughout serves to bring together relevant material under each main topic, and it is hoped that the device of combining author index and bibliography will further facilitate reference to particular studies.

The Imperial Bureau of Animal Breeding and Genetics is greatly indebted to Dr Anderson for the preparation of this technical communication, and joins with him in gratefully acknowledging the assistance of those authors who kindly allowed their illustrations and diagrams to be reproduced here, as well as in expressing appreciation of the help of Dr R. Daubney, Director of Veterinary Services, Kabete, Kenya, in according permission to the author to undertake this review.

J. E. NICHOLS

CONTENTS

PART I

PAGE

CHAPTER 1. INTRODUCTION 1

CHAPTER 2. THE SEMEN OF ANIMALS 1

THE SEMEN OF THE BULL 1, 136

Volume, 2, 136; Motility, 2, 136; Number of Spermatozoa, 3, 136; Hydrogen-ion Concentration, 3; Decline of Motility of Stored Semen, 4; Morphology, 5; Semen Collected by Massage, 7; Effect of Exercise on Semen, 8; Relationship between Different Semen Characteristics, 8, 136; Character of Semen and Fertility, 9, 136.

THE SEMEN OF THE RAM 12

Volume, 12; Number of Spermatozoa, 12; Motility, 13; Hydrogen-ion Concentration, 13; Longevity, 13; Morphology, 13; Effect of Method of Collection, 15; General, 15; Fertility, 16.

THE SEMEN OF THE STALLION 19

Volume, 19; Number of Spermatozoa, 19; Motility, 19; Morphology, 19; General, 20.

THE SEMEN OF THE BOAR 20

Volume, 20; Number of Spermatozoa, 20; Morphology, 21; Hydrogen-ion Concentration, 21; General, 21.

THE SEMEN OF THE DOG 22

THE SEMEN OF THE FOX 22

THE SEMEN OF THE RABBIT 22

THE SEMEN OF THE FOWL AND TURKEY 22

CHAPTER 3. FACTORS AFFECTING SEMEN PRODUCTION 23

TESTIS 23

ACCESSORY ORGANS 25

PITUITARY 28

THYROID 28

TEMPERATURE 29

Seminal Degeneration in the Bull 30

Seminal Degeneration in the Ram 31

Effect of Heat on Fully Formed Spermatozoa 33, 136

LIGHT 34

NUTRITION 34

SEASON 39

Ram 39

Bull 42, 136

OTHER CONDITIONS 42, 137

Dipping and Shearing, 42; Footrot, 42; Abscesses, 43; Trauma, 43; Toxins, 43; Altitude, 137.

Clinical Abnormalities of the Genitalia 43

The Bacteriology of Semen 44

PAGE

FREQUENCY OF COPULATION AND EJACULATION 45
Bull 45, 137
Ram 47
Stallion 48
Boar 48

SEXUAL BEHAVIOUR 49, 137

CHAPTER 4. PHYSICO-CHEMICAL PROPERTIES OF SEMEN 52
Osmotic Pressure 52
Buffering Capacity 52, 137
Electric Charge 53
Electro-conductivity 53
Inorganic Constituents 54
Organic Constituents 57
The Contribution of the Different Accessory Glands to the Semen 60

CHAPTER 5. PHYSIOLOGY OF SPERMATOZOA 62

GENERAL—
Osmostic Pressure 62
Movement 63
Resistance 63
Agglutination 64

EFFECTS OF TEMPERATURE 64
Optimum Temperature 66

DILUTION 67
General Principles 67
Electrolytes and Non-electrolytes, 68; Nutrients, 69.
Fluids for Dilution and Storage 69
Preparation of Diluting Fluids, 69; Sulphate and Tartrate Diluents, 69; Phosphate Diluents, 70; Sodium Chloride, 70; Glucose, 70; Egg-Yolk Phosphate and Citrate, 71; Gelatin, 71; Blood Serum, 72; Tissue Extract, 72; Other Substances, 72
Use of Diluents 73
Quality and Dosage of Gelatinised Sperm, 73.

METABOLISM 74, 137
Respiration 74
Glycolysis 76
Interrelation of Respiration and Glycolysis 80
Hydrogen-ion Concentration 80

VITALITY IN FEMALE GENITAL TRACT 82

MORPHOLOGY 83

FERTILITY 84, 138

CHAPTER 6. STORAGE AND TRANSPORT OF SEMEN 86

PRINCIPLES 86

BULL SEMEN 89, 138

RAM SEMEN 91

STALLION SEMEN 92

SEMEN OF OTHER ANIMALS 93, 139
Boar, 93, 139; Dog, 93; Rabbit, 94; Cock, 94.

PAGE

CHAPTER 7. EXAMINATION OF SEMEN 94

Physical Properties 94

Motility 95

Enumeration of Spermatozoa 95, 140

Hydrogen-ion Concentration 96

Resistance 96

Morphology 96, 140

Preparation of Slide, 96 ; Staining Methods, 97, 140 ; Biometrical Examination, 98.

Metabolism 98

Examination for Abnormal Elements 99

Clinical Examination of Males 99

PART II

CHAPTER 8. THE ARTIFICIAL INSEMINATION OF ANIMALS 100

ADVANTAGES AND USES 100

Limitations and Objections 101

Use in Colonies 102

ARTIFICIAL INSEMINATION OF CATTLE 103

Artificial Insemination Associations 103, 140

Use in Different Countries 104

Russia, 105 ; Denmark, 105 ; Italy, 105 ; United States, 105 ; Great Britain, 106 ; Kenya, 107 ; Palestine, 109 ; Other Countries, 109.

Conception Rate and Factors Influencing it 110, 140

Comparison with Natural Mating, 111 ; Insemination in Different Herds, 112 ; Time of Insemination, 112 ; Age of Cows, 112 ; Site of Injection, 113, 141 ; Range Cattle, 113 ; Gelatinised Semen, 113.

ARTIFICIAL INSEMINATION OF SHEEP 113

Application in Different Countries 113

Russia, 113 ; Kenya, 113 ; Romania, 114 ; South Africa, 114 ; Australia, 114 ; Argentina, 115.

Factors Affecting Insemination Results 115

Season, 115 ; Time of Insemination, 115 ; Number of Inseminations, 116 ; Gelatinised Semen, 116.

ARTIFICIAL INSEMINATION OF HORSES 117

Time and Number of Inseminations 117

ARTIFICIAL INSEMINATION OF OTHER ANIMALS 118

Pig, 118 ; Dog, 119 ; Fox, 119 ; Rabbit, 119 ; Poultry, 119.

PART III

CHAPTER 9. THE TECHNIQUE OF ARTIFICIAL INSEMINATION 120

GENERAL 120

Preparation and Cleaning of Apparatus, 120 ; Handling and Examination of Semen, 121 ; Selection of Females for Insemination, 121 ; Capsules for Gelatinised Sperm, 121.

CATTLE 122, 141

Apparatus, 122 ; Control of Bull and Cow, 123 ; Preparation of Apparatus, 123 ; Collection of Semen, 124 ; Handling and Examination of Semen, 124 ; Dilution of Semen, 124 ; Selection of Cows for Insemination, 125 ; Insemination, 125, 141 ; Storage of Semen, 126.

SHEEP 126

Apparatus, 126 ; Control of Ewe and Ram, 127 ; Preparation of Artificial Vagina, 127 ; Collection of Semen, 127 ; Handling and Examination of Semen, 129 ; Dilution of Semen, 129 ; Selection of Ewes for Insemination, 129 ; Insemination, 129 ; Storage of Semen, 129.

PAGE

HORSE 129
Apparatus, 129; Control of Stallion and Mare, 130; Preparation of Apparatus, 130; Collection of Semen, 130; Handling and Examination of Semen, 131; Selection of Mares, 131; Insemination, 131; Storage of Semen, 131.

PIG 131
Apparatus, 131; Collection, 132; Insemination, 132.

DOG 132

FOWL AND TURKEY 13

OTHER ANIMALS 133

CHAPTER 10. MANAGEMENT OF SIRES FOR BREEDING 134

SUPPLEMENT 136

BIBLIOGRAPHY 142

ILLUSTRATIONS

FIG. FACING PAGE

1. Spermatozoa from 4 bulls of good fertility 4
2. Spermatozoa from 4 bulls with reduced or destroyed fertility 5
3. Abnormal spermatozoa per 1000 for dairy bulls . . . . . *page* 7
4. Types of abnormal ram spermatozoa 13
5. Relation between time of ovulation, distribution of sperm at Fallopian tube and fertility . *page* 85
6. Diagram of new-type artificial vagina . . . . . „ 122
7. Service crate 122
8 and 9. Preparation of artificial vagina (cattle) 123
10. Collection of semen (bull) 123
11. Insemination (cow) 123
12. Collection of semen (ram) 123
13. Position of the genital organs of the bull and method of manipulating them 124
14. A ewe in the service crate 126
15. Gunn's ram restraint table showing top vertical 127
16. The same showing top horizontal 127
17. Side elevation of Gunn's ram restraint table . . . . . *page* 128
18. Missouri-U.S.D.A. model artificial vagina for the horse . . . . . „ 130
19. Apparatus for the collection of boar semen 132

PART I

CHAPTER 1. INTRODUCTION

In natural service semen is deposited in the female genital tract by the male at the time of copulation. With artificial insemination, the male is caused to ejaculate, not into the female, but into some form of receptacle and the semen can then be introduced into the female genital tract by instruments. Artificial insemination is conducted in accordance with thoroughly well-established physiological principles and when properly performed there is nothing in the process which is harmful to either male or female or to the progeny.

The practice of artificial insemination has stimulated a very great amount of work on problems of male and female reproduction. Its use has led to the evolution of standards for the assessment of male fertility, and while the solution of this problem has not yet been finally attained, considerable progress towards this result has been achieved.

The main value of artificial insemination, however, lies in its use as a weapon for the improvement of livestock. There is little doubt that it has a very considerable potential value for this purpose and that increasing use will be made of it in many countries as time goes on. Technical advances in recent years permit a much greater utilization of this method of breeding than is at present practised or even contemplated.

Its present use, apart from its value for the control of genital disease, is largely in inverse ratio to the quality of stock and the availability of good sires in different countries. The growing realisation of its genetic and economic possibilities is now increasing its use in highly developed animal industries, as is illustrated by its adoption for dairy cattle in Denmark and the United States. At the present day artificial insemination seems to hold most promise for cattle breeding. It should prove invaluable for the replenishment and improvement of the severely depleted European herds after the war. It will also provide a means for the improvement of cattle in the Colonial Empire, which probably cannot be achieved in any other way.

CHAPTER 2. THE SEMEN OF ANIMALS

The Semen of the Bull

The ejaculate of a normal bull of good fertility is an opaque, whitish, or whitish-yellow fluid of milky or milky-creamy consistency. Usually, the thicker and more cream-like the ejaculate is, the higher is the concentration of spermatozoa, and conversely, a thinner, more watery, less opaque fluid of a bluish-white colour contains a smaller concentration of spermatozoa. Occasionally the ejaculate may be of a greenish-yellow colour.

Although the appearance of the ejaculate depends mainly on the number of spermatozoa present, there are exceptions, and a few ejaculates which are milky in appearance contain few or no spermatozoa. Cells, such as giant cells, produced in degenerative conditions of the testes, may make the semen opaque. Overwork may cause the semen to become thin and watery, but one of the main causes of changes in the appearance and physical properties of the semen is abnormality in the genital organs. The type and degree of alteration in the semen depend on the actual disease process and the portion of the genital tract affected. The ejaculate from bulls with chronic epididymitis is seldom normal in appearance (Anderson, 1939*b*, 1940). In the initial stages of this disease the semen becomes thinner, less milky and more watery, and may eventually consist of thick mucus or a watery fluid with lumps or shreds of mucus. Tough viscous semen may indicate catarrhal conditions of the accessory genital organs (Lagerlöf, 1934). Large purulent floccules may have originated from purulent inflammation of the seminal vesicles.

The viscosity of semen from normal, fertile, healthy bulls, as measured by Denning and Watson's (1906) method, is directly related to the concentration of spermatozoa, the higher the concentration the greater being the viscosity (author, unpublished results). Within the viscosity range in these observations, there was no relation between viscosity and motility. Motility is, of course, affected when the viscosity is abnormally high.

Volume.—The average volume of the ejaculate is about 4 ml. ; it may range from approximately 0·5 ml. to 12·0 ml. The amount varies considerably in the same bull from time to time, and in different bulls. Herman and Swanson (1941) gave the volume of 334 ejaculates from 50 bulls as from 2·5 to 5·5 ml., with a mean of $4{\cdot}38 \pm 1{\cdot}02$ ml. (s.d. = 1·86 ml.). In one series of 241 ejaculates from 6 normal bulls the mean volume was $4{\cdot}40 \pm 0{\cdot}13$ ml. (s.d. = 2·1 ml.), and in another series of 195 ejaculates the mean volume was $3{\cdot}53 \pm 1{\cdot}35$ ml. (s.d. = 1·89 ml.) (Anderson, 1941*a*). Highly significant differences have been noted between bulls in volume of ejaculate (Erb, Andrews and Hilton, 1942). (*See* p. 136.)

There are indications of possible breed differences, dairy bulls having given larger ejaculates than beef bulls, and Friesians larger than Ayrshires (Anderson, 1941*a*). Lagerlöf suggested that the size of the ejaculate may be related to the size of the bull, as Herman and Swanson (1941) have observed. The volume is usually smaller in young bulls than in adult bulls (Anderson, 1940). Herman and Swanson, however, found that the volume of semen did not vary directly with the age of the bull, and that a few old bulls that were small gave much less semen per ejaculate than younger, larger bulls. The effect of age can be noted when the same bulls are under continuous observation over a period of years. Under these conditions it has been found that the volume has been maintained fairly constant over a period of 5 or 6 years, but it may decrease somewhat as the bull grows older (Anderson, 1941*a*). A second ejaculate collected shortly after the first is usually rather larger (Anderson, 1941*a*). Davis and Williams (1939) noted a slight increase in the volume of the second ejaculate, with little difference between the second and third ejaculates ; individual differences in the bulls were marked.

Lagerlöf has noted that the amount of semen is usually normal in a bull with impaired fertility. In epididymitis, however, the amount is usually smaller than normal.

Motility.—The motility of spermatozoa from normal, fertile bulls is usually high (Anderson, 1940, 1941*a*, 1942*a*). In one series of 456 ejaculates only 3% had a motility of less than 70% ; in another series of 254 ejaculates, 11% had a motility of less than 70% ; and in a third series of 221 ejaculates the mean motility was $73 \pm 1{\cdot}8\%$ (s.d. = 26·7 ; C.V. = 36%). Davis and Williams (1939) found the mean motility of 264 ejaculates to be $73{\cdot}7 \pm 0{\cdot}72\%$ (s.d. = 13·5 ; C.V. = 18%). Herman and Swanson (1941) examined 256 ejaculates from 10 bulls. For most bulls a large percentage of spermatozoa had the power of vigorous motility at ejaculation. They noted that the initial motility varied less than any other characteristic of semen. There was a striking difference between good and poor bulls. Bulls with questionable breeding records were occasionally observed to give high motility ratings. On the other hand, not all bulls which produced semen of good initial motility were of high fertility. Initial motility is considered one of the best signs of viability of sperm (Davis, 1938 ; Herman and Swanson, 1941). (*See also* p. 136.)

Considerable variation does occur in motility even in semen from the best bulls, and it is not uncommon to get specimens of poor or bad motility, from time to time, from perfectly normal, fertile bulls.

Highly significant differences have been noted between bulls in initial motility (Erb, Andrews and Hilton, 1942). Occasionally motility in the first ejaculation is poorer than in the second or third ejaculates. (Also noted by Davis and Williams.)

Motility tends to be poorer when other semen characteristics depart from normal. It is seriously affected by abnormal conditions of the genital organs. In epididymitis it is either poor or absent. In atrophy or hypoplasia of the testes it is much reduced. Lagerlöf (1934), who collected the semen from the vagina, noted the harmful effect of abnormal testicular conditions on the motility, *e.g.* (1) in infectious changes, when spermatozoa were found, the motility was poor or absent, (2) in hypoplasia, the motility was variable, and (3) in degenerative changes, it varied from good to bad depending on the degree of the degeneration.

Donham, Simms and Shaw (1931), who also collected semen from the vagina, found a definite correlation between motility and fertility. They stated that semen which contains less than 90% of active spermatozoa should be regarded as abnormal, since it does not ensure satisfactory fertilisation. Anderson (1941*a*), however, did not find any difference in fertility when the motility varied from 70% to 100%. Swanson and Herman (1941*a*) observed that initial motility was not correlated with the fertility, except in samples of very poor initial motility which were of poor fertility.

The estimation of motility, although of great practical value, is largely subjective. There is a need for an exact measurement to replace the estimation of motility. It is possible that the respiratory rate of spermatozoa (Walton and Edwards, 1938) or the *p*H change (*see* p. 9) may prove of some use for this purpose. For practical purposes of artificial insemination, 70% motility has been regarded

by the writer as the arbitrary border line, and ejaculates of less than 70% are not used for insemination, although it is quite possible that ejaculates of lesser motility would prove fertile. Herman and Swanson (1941), for example, found that semen of very low motility was effective in producing conception ; one of their fertile samples of semen rated a motility of 1, and 4 samples rated 2, at the time of insemination.

Number of Spermatozoa.—The average number of spermatozoa per mm.^{3} is about 600,000 to 1,000,000, but a wide range of variation occurs even in fertile bulls. Lagerlöf (1934) found that the number varied between 300,000 and nearly 2 million per mm.^{3} with an average of 800,000. Milovanov (1934) gave the average as 1,000,000 and the range as from 300,000 to 3 millions per mm.^{3} Davis and Williams (1939) noted a range of from 8000 to nearly 2 millions, with an average of 734,000. Herman and Ragsdale (1939) found a range of from 360,000 to 1,950,000. Anderson (1940, 1941*a*, 1942*a*), in several series of observations on fertile bulls, found an average of from about 600,000 to 900,000 per mm.^{3} with a range of from 50,000 to nearly 2 millions ; one series of 171 ejaculates had a mean of 648,540$\pm$3430 (s.d. = 448,680 per mm.^{3} ; C.V. = 70%). Highly significant differences have been noted between bulls for concentration of sperm per mm.^{3} (Erb, Andrews and Hilton, 1942). The mean for 4 bulls was 914,000. (*See also* p. 136).

The total number of spermatozoa in an ejaculate, depending on both the volume of the ejaculate and the concentration of spermatozoa, is a highly important factor in male fertility, to which more attention will be given in future as the need arises for obtaining the maximum from bulls. The data in Walton and Edwards' (1938) " exhaustion test " showed that the total number of spermatozoa per 10 collections ranged from 2880 million to 32,140 million, with an average of 14,000 million (from each of 14 bulls). In single collections a range of from 616 million to 11,000 million, with an average of 5400 million has been found (Anderson, 1941*a*). Highly significant differences have been noted between bulls for total sperm ; the mean for 528 monthly values for 4 bulls was 3039 million (Erb, Andrews and Hilton, 1942).

Lagerlöf noted the effect of disease on the concentration of spermatozoa : (1) with degenerative changes in the testes, the number of spermatozoa was almost normal when the changes were slight ; when the changes were marked the number was much reduced or spermatozoa were absent ; (2) with infectious changes or fibrosis of the testes spermatozoa were, as a rule, absent or present in small numbers ; (3) with hypoplasia of the testes spermatozoa were absent or present in very small numbers, usually 50,000 to 150,000 per mm.^{3} Spermatozoa are few or absent in epididymitis and in atrophy and hypoplasia of the testes (Anderson, 1940, 1941*a*). When the testes appear to be markedly abnormal on clinical examination, it is likely that the sperm is also affected. On the other hand, spermatozoa may be absent when the testes and epididymides show no detectable abnormalities.

Insufficient work has been done to permit a statement of the approximate number of spermatozoa required for conception. It is moreover unlikely that it will be possible to make any hard and fast rules on this subject for the number of spermatozoa required depends on several factors, which include the activity of the spermatozoa, and the time interval between insemination and ovulation (*see* p. 85; Walton, 1938*b*). It would, however, be of considerable assistance to cattle breeding associations to know the approximate number of spermatozoa required for conception, to allow maximum use to be made of semen. Kufarev (1935) gave the optimum of undiluted semen as 0·5 ml. containing an average of 300 to 400 million spermatozoa ; such semen has given 65% to 72% pregnancies. Neumann (1935) found that quantities of from 0·5 to 2 ml. were most effective for cervical insemination ; quantities in excess of 2 ml. did not increase the percentage of fertility. Kozlova (1937) found no difference in the calving percentage of cows inseminated with 0·1, 0·2, or 0·5 ml. of undiluted semen high in spermatozoa, and cows served naturally. Kozlova (1935) showed that a dose of 0·2 ml. inseminated into the cervix gave as high a percentage of calving as 4 ml. injected into the vagina. Herman (1939) found that the services required for conception were practically the same for semen doses of 0·4 to 6·0 ml. Herman and Swanson (1941) found practically no difference in the conception rate (930 inseminations) with a dose of 0·4 ml. and upwards. Anderson (1941*b*) found that different doses (0·5 and 1 ml.), and different dilutions (up to $\times$4) gave very similar results. This question is obviously related to the number of sperm required for conception and has not yet been fully worked out. Maruškin and Sivokonj (1939), using gelatin capsules containing 150×10^6 sperm, obtained 89% calving. Salisbury *et al.* (1943) in co-operation with the New York Artificial Breeders' Co-operative are diluting all semen at a rate determined by the count ; the semen has been diluted at a rate varying from 1 : 2 to 1 : 14 and comparable rates of conception have been obtained.

Hydrogen-ion Concentration.—One of the earlier estimations of the *p*H of bull semen was made by Webster (1932), who found that almost all specimens lay between 7·0 and 7·5. Later investigations

by Webster (1939*a*), using the Cambridge potentiometer, indicated a range of 6·0 to 7·5. According to Milovanov (1934), the *p*H is usually acid, 6·5 to 6·8, sometimes even 5·5, but with an increase in the amount of the accessory secretions may become neutral or even alkaline, 7·0 to 7·5. Davis (1938) found that the *p*H varied widely as between semen collected by massage of the seminal vesicles and the ampullae of the vasa deferentia, and by the artificial vagina ; by the former method, which yields a relatively greater amount of accessory secretions, a *p*H of 7·5 to 8·0 was not uncommon, while for the latter method the *p*H was ordinarily below 7·0. Šergin (1935), using a quinhydrone electrode, obtained a *p*H of 6·74 and Hatziolos (1937) a *p*H range of from 6·39 to 7·81, with a mean of 6·897±0·107. Davis and Williams (1939), using a quinhydrone gold electrode, obtained a *p*H which ranged from 6·18 to 8·31, with the mean at 6·99. Anderson (1942*a*) found a mean *p*H of 6·73±0·020 (s.d. = 0·300 ; C.V. = 5%), for the semen of 11 fertile bulls. The individual differences in this series were just significant. Herman and Swanson (1941) stated that they found little difference between the *p*H values of the semen of bulls of good and poor fertility. The *p*H was practically the same for the more fertile bulls (*p*H 6·47) and the questionable bulls (*p*H 6·50), but was slightly higher for the poorer breeders, this being mainly due to 2 bulls which produced poor quality semen. They noted that the *p*H of semen showed the least absolute variation next to the initial motility rating. Erb, Andrews and Hilton (1942) noted highly significant differences in the *p*H of semen of different bulls.

Webster (1939*a*) in *p*H tests on bull semen, observed a relationship between *p*H and fertility. He suggested that the probably fertile range is from 6·0 to 7·5, with samples between 7·0 and 7·5 of very doubtful low fertility. The *p*H of the semen of abnormal bulls was investigated by Anderson 1942*b*). An alkaline reaction was characteristic of typical cases of epididymitis, and of bulls with small testes. The alkalinity was associated with a decrease in the concentration or absence of spermatozoa. In clinically normal bulls semen of poor quality was either about the neutral point or alkaline.

When 2 ejaculates are collected, one after the other, from fertile bulls, the second ejaculate is usually more acid than the first, and this change in reaction is, in general, associated with the better quality of the second ejaculate in that there is a greater concentration of spermatozoa of higher motility (Anderson, 1941*a*, 1942*a* and *c*). However, the concentration of spermatozoa may decrease from the 1st to the 3rd ejaculate, as Davis and Williams (1939) found ; and in this case the mean *p*H decreased from the 1st to the 3rd ejaculate. In sterile bulls, the *p*H of successive ejaculates became increasingly alkaline.

The hydrogen-ion concentration would appear to be of general value for the appraisal of bull semen. An alkaline reaction is characteristic of sterile bulls. In clinically normal bulls semen of poor quality tends to approach the neutral point or become alkaline. The alkalinity of semen is associated with a decrease in the activity and concentration of spermatozoa or with their absence. While characteristically the semen of fertile bulls has an acid reaction, the *p*H range of the semen of fertile bulls may be quite wide, and within this range it is not clear that the *p*H has any especial value apart from its general indication of the other semen properties such as the motility and concentration of spermatozoa.

Decrease in p*H on Incubation.*—A significant relationship has been shown to exist between the change in *p*H after one hour's incubation at 37° C., and other characteristics of the semen of the bull (Anderson, 1944*a*). The actual correlations are discussed later (*see* p. 9). The mean *p*H change was −·302±0·027 (s.d. = 0·318). There were highly significant differences between bulls. The range in the *p*H change was from −·87 to +·36, and the average values for individual bulls varied from −·460 to +·113.

Decline of Motility of Stored Semen.—W. L. Williams (1932) reported that the duration of motility in stored semen was a good index of fertility. Herman and Swanson (1941) determined the duration of a motility rating of 2 or better of semen stored undiluted after gradual cooling to 40° F. Motility was rated according to its vigour at 0 to 5, the best motility being rated 5 and no motility, 0. Although the duration of 2 motility was quite variable it was roughly correlated with the initial motility. The average 2 motility rating for good, questionable, and poor breeding bulls was 71 hours, 56·8 hours and 26·9 hours respectively. They considered that a bull should not be rated on this value alone, however, since many bulls of good fertility averaged less than 56 hours maintenance of 2 motility, and a few were even below the average of poor bulls. Also 2 bulls of the poor and questionable groups averaged well above the 71 hours of the good group. Data from 7 bulls of varying fertility used in the same herd indicated that the average time a 2 motility or better was maintained agreed more closely with the bulls' breeding record than did any other values. Nevertheless, it is clear, as Herman and Swanson stated, that judgment of a bull's fertility by this method must be made with reserve.

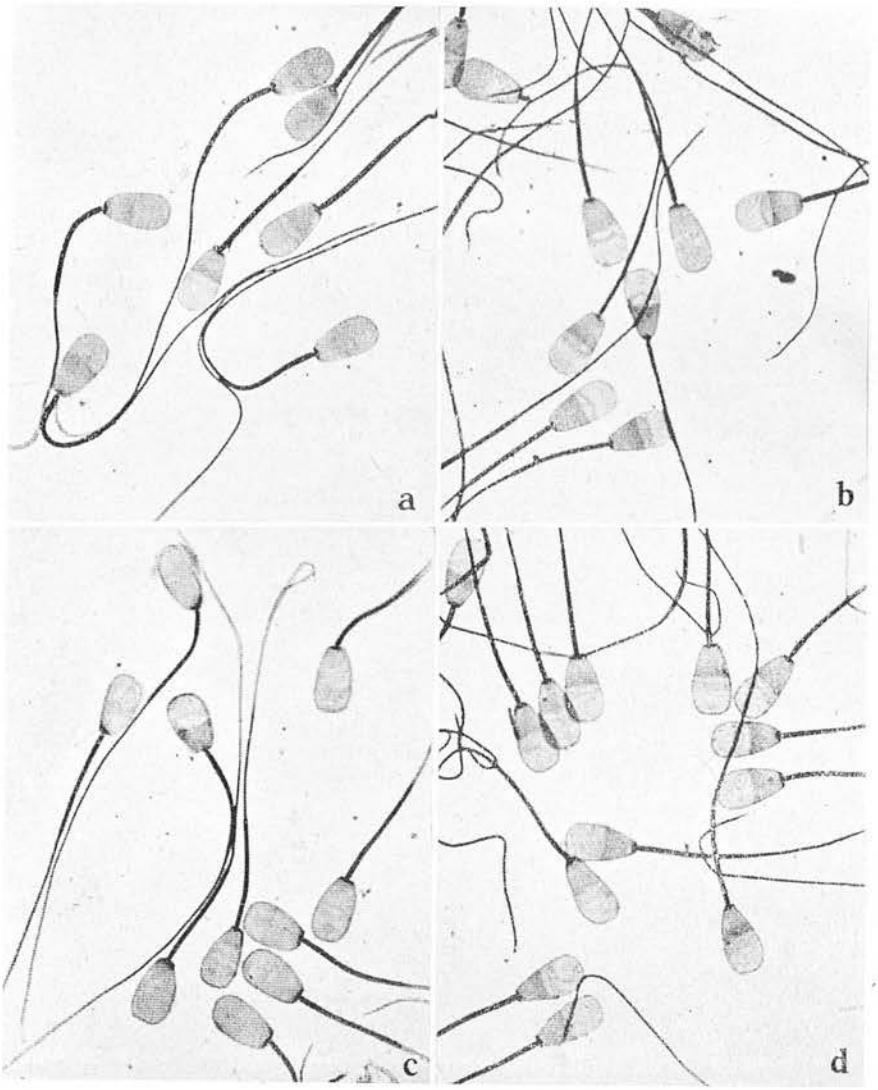


FIG. 1.—Spermatozoa from 4 different bulls of good fertility. ×1000. This and following fig. stained after Williams' method.

(*From Lagerlöf*, 1934)

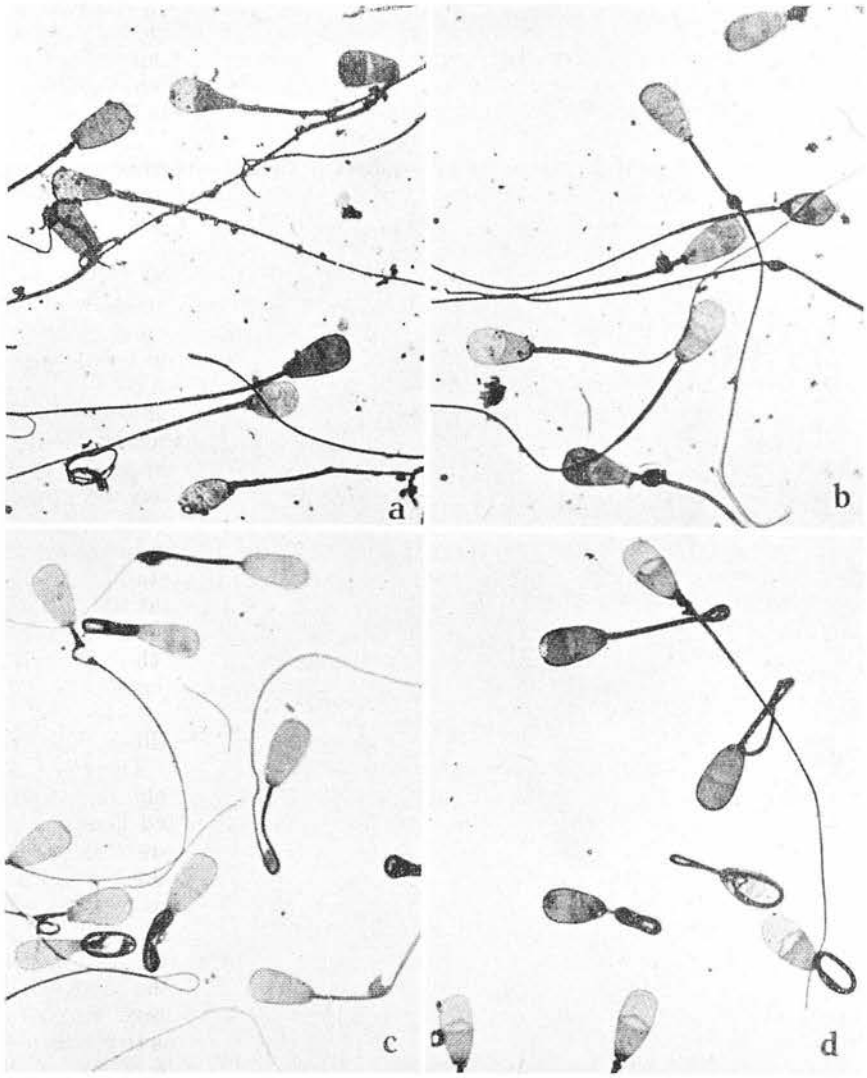


FIG. 2.—Spermatozoa from 4 different bulls with reduced or destroyed fertility. Pictures *a* and *b* show changes in head and disturbance in staining capacity; *c* and *d* show changes in middle piece and tail. Note threadlike middle piece in *c*. A.S.=abnormal spermatozoa.

a=45% A.S. 150,000-450,000 S./mm³. Motility less good. Acute *B. abortus* orchitis in left testis with degeneration of epithelium in right testis.

b=25%-30% A.S. 75,000-125,000 S./mm³. Motility less good. Hypoplasia.

c=40% abnormal heads and 30% abnormal middle pieces and tails. 1,200,000 S./mm³. Motility very bad. Localised degenerative atrophy.

d=15%-20% abnormal heads. 200,000 S./mm³. Motility very bad. Chronic local orchitis with marked degeneration.

(*From Lagerlöf*. 1934)

It appears from results given by Swanson and Herman (1941*a*) that semen from bulls of good fertility should maintain a high percentage of vigorous motility for at least 30 hours in storage undiluted at 40° F. Bulls whose semen maintained good motility for an average of less than 24 hours were of poor fertility. Anderson (1941*a*), however, found no correlation between the fertility from insemination with fresh semen and the motility of this semen after storage for 24 to 96 hours. The stored semen was, however, of high quality, the motility varying from 70% to 100% at the time of insemination. Considerable differences have been observed in the period for which good motility (70% to 100%) was maintained, between different bulls, and even from time to time in different ejaculates from the same bull (unpublished experiments). Highly significant differences have been noted between bulls for survival period ; the mean of 510 monthly samples for 4 bulls was 7·7 days (Erb, Andrews and Hilton, 1942).

Respiration Rate.—Walton and Edwards (1938) found a definite correlation between services per conception (mainly for the period 1 month before and 1 month after the test), and the respiration rate of spermatozoa, in that the higher the respiration rate initially and at 2 hours, the fewer the services that were required for conception.

Morphology.—Lagerlöf (1934) recognised the following main abnormal types of spermatozoa : (1) differing from normal in size, *e.g.* giant and dwarf forms ; (2) with 2 heads or 2 tails ; (3) with abnormally shaped heads ; (4) loose heads ; (5) with abnormalities in the middle piece ; and (6) of disturbed staining capacity. Giant cells are seldom seen in the semen of domestic animals (*see* ram, p. 15). Dwarf spermatozoa may be of normal form, but commonly the basal part of the head is much tapered ; these occur fairly often in bull semen. Changes in form affect the head more often than the tail, particularly the posterior part of the head. There may be a general tapering of the head or of the basal part only, which may become so constricted that it assumes a pear shape. Changes in the shape of the tail are relatively uncommon. Occasionally, there may be ragged connecting pieces. The tail may be bent or coiled to a greater or lesser degree. A very much coiled tail is regarded as indicating that the spermatozoon was dead when ejaculated. Loose sperm heads may be deformed, indicating a disturbance in spermatogenesis. Spermatozoa with abnormal staining capacity (staining too strongly or too faintly) occur relatively often. Lagerlöf (1936) stated that the detrimental agency which caused the disturbance in spermatogenesis, or brought about degeneration in any of the developed spermatozoa, may also have affected spermatozoa other than those which show actual changes under the microscope. Lagerlöf's classification was used by Anderson (1939*b*). (*See* Figs. 1 and 2.)

Herman and Swanson (1941) used the following classification: (1) tailless, (2) coiled tails, (3) pyriform heads, (4) other head abnormalities, and (5) body abnormalities. The most common form encountered was coiled tails. The next most common was tailless, closely followed by pyriform heads. The other head abnormalities—damaged heads, tapering or pointed heads, small and large heads, phantom heads, and undeveloped spermatozoa—were relatively rare. Body abnormalities, including damaged, enlarged, filiform, beaded or shrunken middle piece, double tails, broken neck and protoplasmic drops on the neck, were quite rare. Trimberger and Davis (1942) found that loose heads were the most common abnormality, with broken tails and looped tails ranking next in order.

An important observation which has been the basis of much subsequent work was the association by W. W. Williams (1920) of sperm morphology with sterility in the bull ; the most significant defect was a lessened size or development of nuclear elements. Williams and Savage (1925) considered the morphology of the sperm heads as the greatest single source of information as to the fitness of the cells for reproduction. Breeding inefficiency was not, however, always detectable by sperm abnormalities.

Lagerlöf has shown that the number of pathological spermatozoa is much increased in cases of testicular hypoplasia (average 42%), and in degenerative testicular atrophy (average 36%) (*see* Fig. 2). Chronic changes in the testis and epididymis are in most cases associated with an increase in the percentage of abnormal spermatozoa (Anderson, 1940, 1941*a*). Averages of 23% and 29% respectively have been found in hypertrophy and atrophy of the testis, though considerable variation occurs.

Lagerlöf's data reveal that degenerative changes in the fully formed and previously normal testes caused an increase in the percentage of abnormal types, but the relative incidence of the different types remained much the same. In hypoplasia, on the other hand, the testes are in a state of incomplete development and the incidence of the various types of abnormal sperm was different, there being an increase in loose heads and other abnormalities and a corresponding decrease in the incidence of abnormal heads. This switch in the incidence of different types of abnormalities may indicate an unfavourable condition of the epididymis, possibly associated with depressed secretion of male hormone by the hypoplastic testes.

Hypoplasia is developmental and may be hereditary, as in the Polled Swedish breed of cattle, in which the left testicle is, as a rule, hypoplastic and the right one normal. Degenerative changes can be caused by many conditions, such as fever and toxic influences, as has been noted in a form of foot rot in bulls. Degeneration can usually be caused by raising the scrotal temperature (also noted by Anderson), and this may happen under natural conditions in hot countries. Inflammatory changes are nearly always associated with infection, *e.g.* of *B. abortus* and *B. pyogenes*. *B. abortus* orchitis usually takes an acute form with high temperature and marked swelling of the affected testicle, which distends the scrotum. Service is performed readily, but after a fortnight or so spermatozoa are few or absent in the ejaculate. Prompt removal of the affected testicle is indicated to save the other one (Lagerlöf).

With changes of lesser degree in the sperm, such as poorer motility, although a slight increase in the percentage of abnormal sperm has been noted, the relative incidence of different abnormal types was much the same as in fully fertile bulls ; but with severe inflammatory conditions of the testis and epididymis, the incidence of spermatozoa with abnormal heads increased at the expense of the others (Anderson, 1939*b*). Comparison of the incidence of different types of abnormal forms in Herman and Swanson's data shows that bulls with poor breeding records had a relatively greater number of coiled tails and tailless forms than had bulls with good breeding records.

The maximum number of pathological spermatozoa permissible for good fertility was, until fairly recently, generally considered to be comparatively low. In the bull, Williams and Savage (1927) showed that fertility was diminished when abnormal spermatozoa exceeded 17%. In 50 bulls of good fertility, Lagerlöf (1934) found that the count of abnormal spermatozoa lay between 2·4% and 17·2%, with an average of 10·7%. He believed that when the count exceeds 18% to 19% there is probably a disturbance in spermatogenesis, which can be so serious that impairment of fertility is possible. Anderson (1939*b*) found averages of 8·1% (range 2%-18%), 13·1% (range 2%-31%), and 17·6% (range 2%-66%) respectively in fertile bulls, bulls regarded as of " reduced " fertility, and in sterile bulls. In a further investigation (Anderson, 1941*a*) 6 fertile bulls gave an average of 10·5% with a range of from 4% to 16% ; for 10 other fertile bulls the average was 6·5% with a range of from 3% to 10% ; for 30 other clinically normal bulls with " good " sperm, the average was 10·6% and the range 4% to 20%. Davis, Trimberger, *et al.* (1940) found that the percentage of atypical sperm in the semen from 11 fertile bulls was relatively constant, averaging 18% or less. Swanson and Herman (1940) examined 300 ejaculates from 55 bulls. Bulls of known good breeding efficiency averaged well below 20% abnormal spermatozoa. All bulls known to be of poor breeding efficiency produced more than 20% abnormal sperm. Three sires which were practically sterile had more than 60% abnormal forms, and in four known to be of low fertility abnormal spermatozoa ranged from 23% to 37%.

Herman and Swanson (1941) noted wide variation in the percentage of abnormal spermatozoa. The semen of bulls of good, questionable, and poor fertility averaged 14·2%, 24%, and 45·8% of abnormal spermatozoa. The distribution of bulls of good fertility, according to the number of abnormal spermatozoa, showed that the semen of 4 bulls contained more than 25% of abnormal spermatozoa, and the semen of 2 bulls more than 30%. Only 2 bulls had semen averaging less than 5%. (*See* Fig. 3.) The majority of the abnormal forms, for 3 of the 4 highest bulls, were coiled tails ; the other bull had 16% of pyriform heads. No particular type of abnormality seemed to be associated with reduced fertility. The main distinctions between the spermatozoa of bulls of good fertility and those of poor fertility were the percentage of total abnormal spermatozoa and the uniformity of size of heads. Some bulls with sperm of abnormal morphology, however, were very fertile. Herman and Swanson obtained conceptions in 12 cows with semen containing more than 20% of abnormal spermatozoa and in 2 of them with semen containing over 50% abnormal spermatozoa. Some bulls of low fertility had semen which appeared to have very few morphological defects. Semen with less than 30% abnormalities is not necessarily fertile, because it may be poor in other qualities, which vary independently of morphology. In general, therefore, Herman and Swanson concluded that it appears that the upper limit of percentage of abnormal spermatozoa compatible with good or fair fertility is approximately 30. When the percentage exceeds 30 and approaches 50 it is likely that some pathological condition is present and the breeding efficiency of the bull will be poor. Highly significant differences have been noted between bulls for abnormal sperm (Erb, Andrews and Hilton, 1942) ; the mean of 528 monthly values for 4 bulls was 12·7%.

Herman and Swanson considered the differences in the percentage of abnormal spermatozoa associated with fertility, between their own studies and those of Williams and of Lagerlöf, to be connected with forms with coiled tails. They stated that evidently W. W. Williams (1920) and

Williams and Savage (1927) and Lagerlöf did not consider coiled tails abnormal. However, from Lagerlöf's account, it seems clear that a much coiled tail, or rolled up tail, was considered abnormal. Herman and Swanson found that coiled tails unaccompanied by abnormal heads made up the predominating type of abnormal spermatozoa for some bulls of low fertility.

Trimberger and Davis (1942) studied the morphological characteristics of the sperm of 24 dairy bulls at monthly intervals for a period of several years. The bulls averaged 790 normal cells per 1000, with a range of from 276 to 968 for individual samples, and from 375 to 903 from the lowest to the highest averages for a bull. The breeding efficiency was 57·76% of the cows bred during the experimental period. Bulls with over 900 normal cells per 1000 had significantly better breeding records than those below this high level, found in only 11·4% of all samples. The breeding performance with over 900 normal cells per 1000 was 74·6%, with 851 to 900 normal cells, 63·2%; and with 500 to 850 normal cells, 51·2%. Bulls with less than 500 normal cells per 1000 had very poor breeding records.

Lagerlöf recognised spermatozoa with protoplasmic drops on the neck as "immature." The presence of more than 2% to 3% was regarded as indicating pathological changes in the genital organs

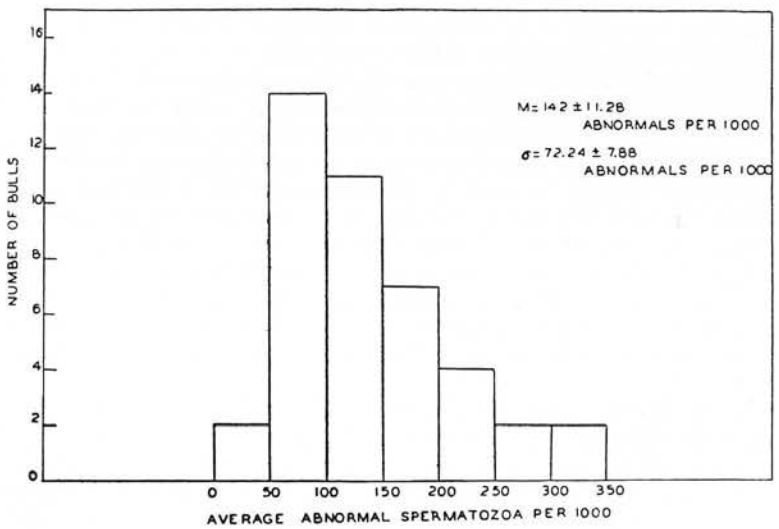


FIG. 3.—Distribution of the average number of total abnormal spermatozoa per 1000 for dairy bulls of high fertility.

(*From Herman and Swanson*, 1941)

and reduced fertility. A large number occurred, as a rule, both with a commencing degenerative process in the seminal epithelium and a regeneration of the same. In hypoplasia they averaged 28% (range 11% to 66%); in degenerative changes the number varied greatly (0% to 66%). They were few in number in bulls of good fertility. About 6% of such sperm were found in cases of epididymitis (Anderson, 1939*b*).

Primordial cells were found by Williams and Savage (1925) in only 3% of cases, and most of these were badly diseased bulls. Their presence to the extent of 1% indicated a grave condition. They considered the presence of epithelial cells, pus, bacteria, extruded cytoplasm, and blood as abnormal.

Head Lengths of Spermatozoa.—Savage, Williams, and Fowler (1927) investigated the relationship between the head length of spermatozoa and fertility. They found that in bulls of good fertility the head length showed normal or almost normal distribution and they maintained that the coefficient of variation of head length provides a good indication of fertility; in normal bulls the coefficient should not exceed 4. Lagerlöf considered bulls with a coefficient of 4 and under as probably fertile, and those with a coefficient of 4·5 and over as of reduced fertility; values from 4 to 4·4 were inconclusive. He found that the coefficient of variation in (*a*) 30 fertile bulls was 3·7, (*b*) 15 of doubtful fertility 4·8, and (*c*) in 30 sterile or almost sterile bulls it was 6·2.

Semen Collected by Massage.—Compared with semen collected by the artificial vagina, semen collected by massage of the ampullae is of lower motility both initially and after storage for 51 hours

at 50° F. Massage specimens tended to have a lower concentration of sperm and to vary in other particulars, especially in their *p*H. *p*H's of 7·5 to 8·0 were not uncommon. There also seemed to be a great danger of bacterial contamination in massage samples (Davis, 1938). The massage method of collection has been used by Kingman (1936), Clapp (1938), and Cole (1938), with successful insemination results. Clapp was able to obtain highly motile sperm in about 90% of the attempts. Kingman (1936) stated that with collection of semen by massage, contamination of the specimen cannot be avoided altogether, but it can be reduced by vigorous washing with warm water. The seminal vesicles are avoided and the ampullae picked up at neck of bladder and milked out. The method has not always been successful but such cases were the exception ; it failed repeatedly in the same individuals. Clapp (1938) stated that the skill of the operator is a main factor in this method, and no injurious effects from massage have been noted. The bull can be used both naturally and artificially without one kind of service affecting the other, except that attempts at artificial collection soon after a natural service may be unsuccessful. The massage method of collection has been mainly used for artificial insemination. It is now a routine procedure that is used regularly. It takes less time ordinarily than to take the cow to the bull on another farm or to breed the cow to a slow serving bull on the same farm and certainly less than to breed several cows naturally. Cole and Winters (1939) stated that the massage method was used very extensively at Grand Rapids, Minnesota, but offered some obstacles in that it was sometimes difficult to teach old bulls to react. The technique of successful massage requires careful study so that the semen will not be contaminated with urine or carry undue quantities of seminal fluid. The particular advantages are (1) semen is obtained easily and quickly after the operator has gained experience and the animal has become trained, (2) no special equipment is required, and (3), what is of most importance, proved bulls that are for some reason or other unable to mount a cow may be kept for service. Henderson (1939) stated that experiments performed at Nebraska indicate that semen collected by massage has poorer keeping qualities. Davis, Trimberger, and Underbjerg (1940) obtained in 400 attempts by the massage method 378 semen samples with very active motile sperm. The average volume was 5-7 ml. and the concentration was 429,000 per mm.^{3}. The *p*H was usually above 7·00. A total of 107 conceptions resulted from 181 inseminations (1·69 inseminations per conception) with semen collected in this way, as compared with 122 conceptions from 188 inseminations with semen collected with the artificial vagina (1·54 inseminations per conception).

Effect of Exercise on Semen. Bartlett and Perry (1939) found that regular exercising of bulls, used in a co-operative breeding project, one hour at $2\frac{1}{4}$ miles per hour, increased the semen output 51%. Kelley (1940) reported that exercise at $2\frac{1}{2}$ miles per hour for 30 minutes per day greatly increased the quality of the semen produced. The motility was 5% to 45% before exercise, and after exercise it was 60% to 100%. Without exercise motility ceased at 3 to 24 hours, but with exercise motility was maintained for 22 to 39 hours. Hamilton and Symington (1939) observed that daily exercise increased the volume of the ejaculate, sperm concentration, and motility.

Relationship between Different Semen Characteristics.—Recently several investigations have been made to determine the normal variation in certain properties of the semen of the bull, and to study the interrelationship between them. (*See also* p. 136.)

Semen samples vary widely in all properties studied. This variation is observed in different ejaculates from the same bulls as well as in those from different bulls. Herman and Swanson (1941) observed the greatest variations in the length of time vigorous motility persisted on storage and in the percentage of abnormal sperm ; variation in the latter was not so great, however, as in the former. Variations in initial motility and *p*H were not great, but semen of high *p*H, 7·00 or higher, was usually of low viability. Swanson and Herman (1941*a*) noted that among separate ejaculates from the same bull, initial motility was roughly correlated with viability in storage. In New Zealand (Blake, 1941) a correlation has been shown between the semen evaluation (based on morphology and motility), and the service record.

The various characteristics may vary apparently independently of each other. Ejaculates of good motility may contain a high percentage of abnormal spermatozoa, and ejaculates of low motility may contain only a small percentage of abnormal spermatozoa (Anderson, 1941*a*). From a critical study of Lagerlöf's data, there was generally a correlation between the concentration of spermatozoa, the percentage of abnormal spermatozoa, and the motility, but this association did not hold in every case. Swanson and Herman (1940) found that, with the exception of semen containing very high percentages of abnormal spermatozoa, 50% or more, there seemed to be no definite correlation between the abnormality count and initial motility or length of survival with good motility. This observation held for variations in semen from the same bull as well as for that from different bulls. Morphological

variations could not be correlated in any significant manner with concentration, volume, viscosity, or *p*H of the semen. Herman and Swanson (1941) stated that the importance of morphology of sperm to fertility seems to be secondary in most cases, *i.e.* regardless of the percentage of abnormal spermatozoa, if the sperm lacks high viability it is of low fertility, but semen high in the percentage of abnormal sperm is often fertile, apparently because it has high viability. However, semen with a high percentage of abnormal forms is usually of poor motility and viability. Semen with less than 30% abnormal sperm is not necessarily fertile, because of the variation in other factors important to fertility, such as sperm motility and viability. Semen may contain sperm of excellent morphology and high initial motility, but which are low in viability. For these reasons Herman and Swanson regarded abnormal morphology or initial motility as of limited importance for the evaluation of semen to be used in artificial insemination.

Davis and Williams (1939) found slight correlations between the volume of semen and the percentage motility, between volume of semen and concentration of spermatozoa per mm.^{3}, and between percentage motility and concentration of spermatozoa per mm.^{3}. A significant correlation has been noted between the *p*H of the ejaculate and the number of spermatozoa per mm.^{3}, in that the greater the concentration of spermatozoa the lower the *p*H (Schneerson, 1936 ; Davis and Williams, 1939 ; Davis, Underbjerg, and Williams, 1940 ; Anderson, 1942*a*). There are also significant negative correlations between the *p*H and volume of ejaculate, and *p*H and motility of spermatozoa (Davis and Williams ; Anderson ; Davis *et al.*). Dougherty and Ewalt (1941) found a correlation (in 645 specimens from 105 bulls) between the *p*H of semen in the first few hours and the motility and viability of the spermatozoa. The lower the *p*H the greater was the concentration of spermatozoa, the higher the degree of motility and the larger the volume of the ejaculate (Anderson). There were, however, exceptions to this inverse relationship. Of a total of 221 ejaculates, 12 had a *p*H in the range 6·31 to 6·90, associated with a mean motility of 38% (range 10% to 60%) and a mean number of spermatozoa of 274,000 per mm.^{3}, and twenty-two ejaculates had a *p*H of 6·91 to 7·20 with a mean motility of 76% (range 70% to 100%), and a mean number of spermatozoa of 436,000 per mm.^{3}. In no instance was a high motility found with a *p*H greater than 7·60. This significant inverse relationship between the *p*H and the concentration of spermatozoa, and the degree of motility, should prove useful for the evaluation of semen. The determination of the *p*H will give an indication of the concentration as well as of the motility of the spermatozoa. There were, however, about 15% of ejaculates for which this negative correlation did not hold, and for which *p*H alone did not give a true evaluation of the semen. The determination of both *p*H and motility, however, as Davis and Williams suggested, should form a sound basis for semen evaluation.

A highly significant correlation has been noted between the change in *p*H after one hour's incubation at 37° C. and the number of spermatozoa per mm.^{3}, and also the initial motility, in that the higher the degree of initial motility, and the greater the concentration of spermatozoa per mm.^{3}, the greater the decrease in *p*H on incubation (Anderson, 1944*a*). There was a highly significant correlation between the change in *p*H and the initial *p*H in that the greater the *p*H change the lower was the initial *p*H. There were some exceptions to these general relationships. The *p*H change is not always large when other semen characteristics are good, as for example a *p*H decrease of less than 0·29 for ejaculates of low initial *p*H, containing a large number of spermatozoa ; conversely, the *p*H change may be large when the motility is poor and the number of spermatozoa per mm.^{3} is small. The *p*H change, however, was not great when the initial *p*H was 7·11 or higher. In general, the *p*H change provides an exact measurement of semen which agrees closely with the measurement of initial *p*H, the number of spermatozoa per mm.^{3} and the degree of motility. The *p*H change stands in a similar relationship to the motility and concentration of spermatozoa as the initial *p*H, for the *p*H change and the initial *p*H are positively correlated, ~~and both bear a negative correlation to the motility and concentration of spermatozoa~~. negatively The *p*H change is therefore confirmatory of the initial *p*H. It is suggested that the determination of the initial *p*H and the *p*H change after one hour's incubation may provide a useful and accurate evaluation of the semen of the bull.

Character of Semen and Fertility.—Since male fertility is determined from the result of mating between male and female, it is necessary, when investigating the relationship between semen characters and fertility, to allow for the female factor. Female fertility often varies from herd to herd, according to various conditions, which include management, feeding, and genital disease. The actual fertility of bulls from herd to herd as expressed by the number of services or inseminations required per conception is therefore purely relative, because of the variation in female fertility between the herds. In an investigation on artificial insemination of cattle, for example, the number of inseminations per conception differed greatly in 3 herds because of genital disease, although according to the

criteria used for assessment of semen, there was little difference in the quality of the semen used on the different farms (Anderson, 1938*a*). Under optimum conditions of female fertility a certain quality of semen might be associated with a fertility level of, say, 1·5 inseminations per conception, but under sub-optimum conditions it might be 2, 2·5, or more inseminations per conception.

Problems of male fertility in cattle should preferably be studied on a large number of females kept, as far as possible, under optimum conditions. This can be done with the aid of artificial insemination, making the requisite examinations on the semen actually used for insemination. It is difficult or impossible to obtain or determine exactly what are optimum conditions, but uniformity of conditions can be obtained by carrying out inseminations in the one herd which is sufficiently large to allow adequate numbers of females being inseminated with different types of semen. Much valuable knowledge will also, no doubt, be gained through the use of artificial insemination in cattle breeding associations. With the use of a few bulls on a large number of cows on many farms, differences due to the female stock and the conditions under which they are run would be recognised and minimised to an increasing extent as time goes on and the breeding efficiency of members' herds increases.

Fertility in the bull may be estimated from (1) previous breeding records, (2) clinical examination of the genital organs, and (3) examination of the semen. Breeding records refer to the past history of an animal and are therefore of value only for that purpose. When based on calvings they refer to a period ante-dated by at least 9 months. A closer estimate may be attempted by basing fertility on cows " holding." But even when this method is reliable, there is still an intervening period during which fertility may have become affected. A bull may become sterile within a very short time, as for example following the specific coital infection of cattle prevalent in Kenya, or indeed through any condition causing degeneration of the epithelium of the seminiferous tubules, which Lagerlöf stated can occur in a period of a few days.

Clinical examination of the genital organs is a useful adjunct only. It is possible to detect changes in the size, shape and consistency of the testes, epididymes, and in some cases the seminal vesicles, and such abnormalities are probably associated with alterations in fertility, but sterile bulls and bulls of low fertility may have, as far as can be detected clinically, perfectly normal genital organs. It is therefore on the examination of the semen that judgment must be based.

Measurements of semen may be divided broadly into two classes, (*a*) quantitative measurements, which include the estimation of the concentration of spermatozoa per mm.^{3}, the volume of the ejaculate, the total number of spermatozoa per ejaculate, the respiration rate of spermatozoa, the *p*H of semen, and the *p*H change, and (*b*) measurements of activity, viability, and resistance, which are at best semi-quantitative (Walton and Edwards, 1938). The recognition of different morphological forms is essentially a qualitative estimation, expressed quantitatively. Clearly, the former class is the more valuable, in so far as it provides exact repeatable standards.

Some of these measurements are related to the accessory secretions rather than to the spermatozoa. There is, however, on the whole, a good correlation between accessory gland and testicular function. The initial *p*H of semen, for example, is probably due mainly, if not entirely, to accessory gland function rather than to the metabolic activity of the sperm, but the reaction of the semen is usually associated with the concentration and activity of the sperm, acidity of semen being usually associated with the presence of numerous very active sperm in the ejaculate. It is possible, however, for accessory gland and testicular function to vary independently of each other and occasionally, for example, an acid semen may be of otherwise poor quality.

Other measurements are more directly related to the spermatozoa themselves, and these are clearly of greater importance for fertilization. Activity and viability are of primary importance, but so far these estimations are only approximate. Determination of activity may be placed on a quantitative basis by its high correlation with the respiration rate, initial *p*H of semen and the *p*H change. Since, however, semen of poor motility, and containing a high percentage of abnormal sperm, may still show considerable metabolic activity, the motility examination cannot be dispensed with. It seems to the writer that the viability of sperm is at present likely to be limited to some extent by the inadequacy of the present storage methods, as well as by inherent qualities in the sperm themselves. It is possible that estimation of functional activity rather than morphological variation will provide a better evaluation. Present work would seem to indicate that most promise lies in the study of the metabolism of spermatozoa.

The stress laid upon one or other of the available evaluations varies among the different workers. Lagerlöf (1934) stated that if the number of spermatozoa in repeated examinations is low, *e.g.* below 200,000 per mm.^{3}, if pathological spermatozoa amount to 20% or more, if large numbers of immature sperm are present, if the motility on several examinations is clearly reduced, and if pathogenic bacteria

can be demonstrated in the semen, then there is reason to assume that such serious disturbances exist in spermatogenesis, or in the function of the accessory genital organs, that the fertility of the bull is reduced or is altogether absent. Walton and Edwards have found that the respiration rate of sperm gave a better indication of fertility than any of the other measurements. No other single criterion has been found adequate to determine small differences in fertility (Milovanov, 1934 ; Walton and Edwards, 1938 ; Davis and Williams, 1939 ; Anderson, 1939*b*, 1940, 1941*a* ; Herman and Swanson, 1941). Dougherty and Ewalt (1941) found little correlation between breeding efficiency and laboratory findings such as the amount of the ejaculate, sperm-cell count, motility, *p*H of semen, and percentage of abnormal sperm. Swanson and Herman (1941*a*) noted that among different bulls, properties of semen, such as *p*H, concentration, volume, percentage of abnormal spermatozoa, and initial motility were not correlated with fertility. Bulls of low fertility may apparently produce semen normal in these properties. They found that the property most nearly correlated with fertility was the time of survival with vigorous motility in semen stored at 40° F. There are indications in preliminary experiments that the *p*H change may be of value for this purpose. At present, however, it is necessary to base the estimate of a bull's fertility on as many different criteria as possible, bearing in mind the necessity for the presence in the ejaculate of an adequate number of very active, morphologically normal spermatozoa. (*See also* p. 136.)

In general, it is possible to identify a characteristic type of semen with fertility, sterility, or with " reduced " or " doubtful " fertility. The extreme cases of semen evaluation are those (*a*) in which the semen is so poor that it is useless for impregnation, as for example, absence of spermatozoa, or dead spermatozoa, and (*b*) in which the semen examined by all available criteria is " good " and conforms with that of fertile bulls. In the latter class of ejaculate it is not possible to identify a particular type of ejaculate, and one showing certain features, with a particular degree of fertility. Semen may be judged as perfectly normal by certain criteria, yet the fertility may be low, perhaps due to poor viability of spermatozoa. Some semen, on the other hand, may be exceptionally poor and yet be capable of occasional impregnation. With some bulls, also, there may be a high incidence of abortions following service, or the semen may contain streptococci or other organisms (Williams, W. L., 1920 ; Lagerlöf, 1934). Such cases do not invalidate the available criteria for semen evaluation, but because of them it is necessary to recognise that fertility cannot be guaranteed from a semen examination. Lagerlöf therefore said that when issuing a certificate for a semen examination it should not be stated that the bull is fertile, but mention should only be made of what has been discovered in the examination. Only in cases in which obvious changes are found in the semen, indicating that the bull's fertility is reduced, should mention of sterility be made.

With intermediate types of semen the position is by no means clear. A particular ejaculate, or a series of ejaculates, may fail in respect of one or other criterion and be therefore regarded as below the standard normally associated with fertility. However, a surer diagnosis can be reached when several criteria are determined in a series of examinations. When the concentration of spermatozoa is low, the motility poor, the *p*H high, the viability poor, and the percentage of abnormal spermatozoa high, it is likely that the fertility is reduced or even absent.

An evaluation of a single ejaculate refers to that ejaculate only, so in view of the range of variation that occurs in semen characteristics it is desirable that an estimate of fertility based on semen examination should be done on a number of ejaculates. Walton and Edwards (1938) subjected bulls to an " exhaustion " test, collecting a series of samples from each bull within the course of a few hours, with short intervals between each sampling. Besides providing a number of samples which gave the range of variation and the mean, this method also indicates the bull's potential reserve of spermatozoa. Whether collection of many samples in a short period will give the same results as collection of the same number of ejaculates over a longer period is not known. The latter procedure conforms more closely to the normal usage of bulls. Swanson and Herman (1941*a*) proposed that a bull's fertility should be rated from examinations of at least 5 semen samples collected over a period of at least 2 weeks (determining average survival time with good motility), and that the suitability for use or storage of a sample of semen from a fertile bull can best be determined at the time of collection by examination for motility.

It is, however, sometimes necessary to decide whether or not a particular ejaculate can be regarded as typical of a bull. This is of particular importance when a bull is unwilling to ejaculate more than once when tested. When a single ejaculate, judged by available criteria, is classed as " good," the bull can probably be regarded as fertile, but a poor ejaculate does not by any means condemn the bull. In such cases, two or more ejaculates should be obtained. If the semen is now good, the bull can probably be regarded as fertile, but if there is no improvement, or if the semen is worse, the bull

is to be regarded as of doubtful fertility. In such cases repeated tests, at, say, monthly intervals, should be made to determine if the bull is improving or deteriorating. Some bulls may temporarily produce poor or bad sperm but in time return to normal ejaculates. The extent to which this is possible will depend on the degree of involvement of the testes and accessory reproductive organs.

Prognosis in doubtful or sterile bulls should be made on the breeding history, clinical examination of the genital organs, and semen examination, repeated when necessary at intervals. Lagerlöf regards the prognosis as not very good when the semen does not undergo any essential improvement in 3 to 4 months.

The Semen of the Ram

The semen of the ram has typically a creamy appearance. As in the bull, semen with a thinner consistency and a milky or watery appearance has usually a lower concentration of spermatozoa. When spermatozoa are absent the semen is thin, clear and watery.

The typical ejaculate produced by electrical stimulation consists initially of a small amount (a few drops to 0·25 ml.) of a clear watery fluid, believed to be essentially prostatic fluid; then 1 to 2 ml. of thick semen, creamy in colour and consistency, containing the majority of the spermatozoa; and thereafter a certain amount of watery secretion from the accessory organs, which varies with the number of stimuli applied.

Volume.—The volume of semen ejaculated by the ram is about 1 ml. or rather less. For semen collected with the artificial vagina, Moskovits (1934) gives the average volume as 1 ml. and Milovanov (1934) as from 1 to 2 ml. In some 1200 ejaculates from purebred Merino rams the average volume was $0{\cdot}72 \pm 0{\cdot}10$ (s.d. = 0·32) (Anderson, unpublished data). For semen collected from the dry vagina of ewes, Terrill (1937, 1938) found the average volume to be about 0·90 ml. (range 0·10 to 2·00). The volume of semen collected with the electrical stimulation method is rather larger, 2 to 3 ml. according to Gunn (1936); in a series of ejaculates from purebred Merino rams it was $1{\cdot}29 \pm 0{\cdot}08$ ml. (s.d. = 0·64) (unpublished data).

Gunn found that ejaculation induced by the electrical stimulation method as frequently as once daily neither decreased the volume of the ejaculate nor lowered the number of spermatozoa ejaculated.

The average volume of semen, ejaculated during 30-minute test periods throughout the year, was about 2·5 ml. Individual ejaculations during these periods ranged from nil to 9·0 ml. (McKenzie and Berliner, 1937).

Number of Spermatozoa.—The average concentration of spermatozoa in semen collected with the artificial vagina, or from the vagina of the ewe, is about 2½ to 3 million per mm.^{3}. The data given by different workers are: 2·85 million (Moskovits, 1934); 2 to 4 million (Milovanov, 1934); 3·016 million (range 0·86 to 5·70) (Terrill, 1937); 2·290 million (range 0·83 to 3·71 million) (Terrill, 1938); 3 to 5 million (McKenzie and Berliner, 1937) and $2{\cdot}57 \pm 0{\cdot}16$ (s.d. = 1·32) (Anderson, unpublished data).

The average ejaculate resulting from electrical stimulation contains about 1·5 million spermatozoa per mm.^{3} according to Gunn and co-workers (1942). There is considerable variation in the concentration of spermatozoa collected in this way, and counts of up to nearly 5 million have been obtained (personal observations).

The total number of spermatozoa per ejaculate collected with the artificial vagina is about 2000 million. According to Moskovits it is 2850 million; to Milovanov 2000 to 4000 million; to Terrill (1937) 2913 (range 116 to 7904); to Terrill (1938) 2181 (range 729 to 5194); and to Anderson, 2050 million. That resulting from electrical stimulation is rather higher. Gunn and co-workers found an average of 3½ to 4 thousand million spermatozoa per ejaculate, and up to over 7000 million have been obtained. Since a smaller number of stimulations is usually required to cause ejaculation of a suitable semen sample from a healthy normal ram, and a greater number from a genitally abnormal ram, the total number of spermatozoa ejaculated is divided by the number of stimuli given to provide a figure for the average number ejaculated per stimulation.

McKenzie and Berliner (1937) found that the average total number of spermatozoa ejaculated in a 30-minute test period by groups of Shropshire and Hampshire rams was from 1800 to 9000 million, and from 2500 to 9800 million, respectively. Individual totals ranged up to 27,400 million for this period. After deducting abnormal types of groups of spermatozoa, the average total of normal spermatozoa for this period for Shropshires and Hampshires respectively was from 500 to 8600, and from 2400 to 9500 million. Terrill obtained a figure of 5344 million normal spermatozoa per 30-minute period (range 82 to 17,311) in 1937, and 4493 (range 554 to 11,610) in 1938.

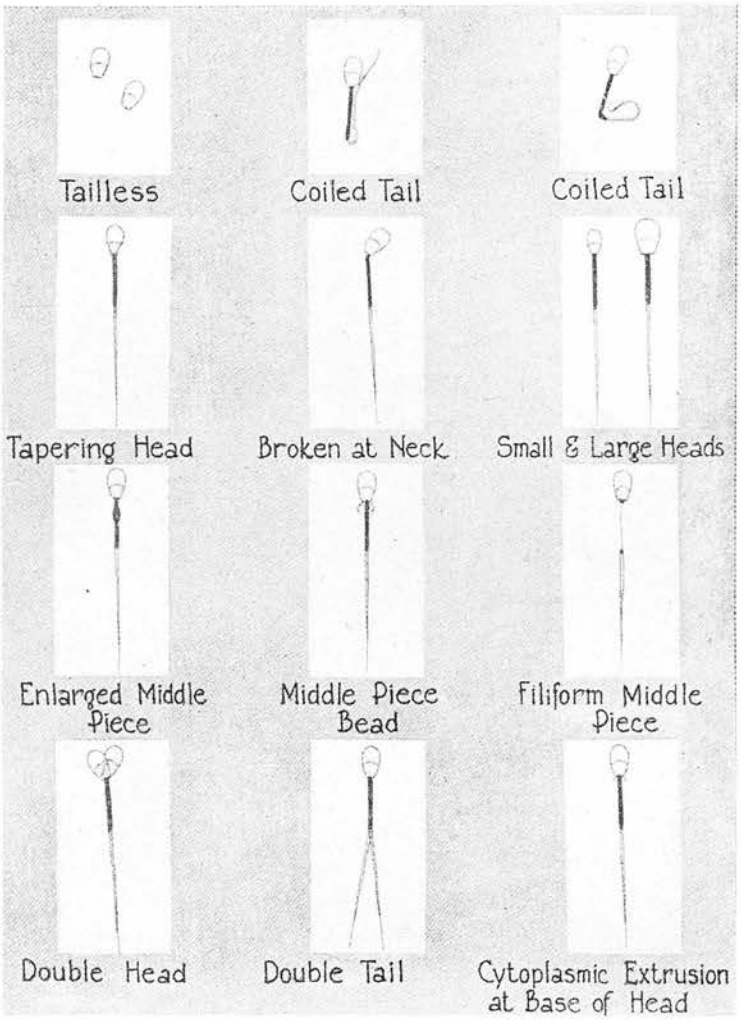


FIG. 4.—Types of abnormal ram spermatozoa.

(*From McKenzie and Phillips*, 1934)

Kuznecov (1934) found that 60 million spermatozoa per dose are necessary for cervical insemination, and Milovanov (1934) showed that about eight to ten times that number are necessary for vaginal or natural insemination. Panyševa (1934) observed little difference with semen diluted up to $\times 16$, using a dose of 0·2 ml., which would mean a dose of about 50 million sperm, assuming there were present 2000 million sperm in 1 ml. of ejaculate. Nagornyĭ (1939) found that dosage from 0·04 to 0·2 ml. had no significant effect on fertilization (it ranged, however, from 72% to 84%). He correlated the dosage of sperm according to quality as determined by resistance R (*see* p. 96), and considered the use of sperm with low resistance unprofitable, since the number of sperm required is high, *e.g.* 8.4×10^8 of diluted sperm if R = 2000; with sperm of high resistance the number can be much smaller, *e.g.* $30\text{-}40 \times 10^6$ if R = 60,000. The fact that the maximal dose (0·7 ml.) is 70 times greater than the minimal (0·01 ml.) clearly demonstrates the inadequacy of dosage by volume. He recommended doses of 0·01 to 0·02 ml. but since small doses are difficult to apply, it is more expedient to use doses of 0·05 ml. with the appropriate degree of dilution.

Milovanov *et al.* (1937) gave the standard dose of sperm required for vaginal insemination as 500×10^6 but stated that this varies from 1200×10^6 to 75×10^6 according to the resistance of the sperm. In Kenya experiments the average number of sperm used for cervical insemination has been about 125 million, and there have not been, on the whole, great differences between the use of diluted and undiluted semen; this question, however, requires further investigation.

Motility.—Terrill in 1937 noted a range of from 0% to 100% motile sperm for 44 rams (mean 58%), and in 1938 from 40% to 100% (mean 73%) in 21 rams. In over 1200 ejaculates from pure-bred Merino rams, 93% showed 100% motility (personal observations).

Habibullin (1938) observed that motility is an inadequate criterion of fertilising ability; in undiluted semen during short periods—up to 12 hours—the curves for motility and fertilising ability ran fairly close, but after 12 hours activity was still maintained at a high level (70%), whereas fertilising ability fell to 32%.

Hydrogen-ion Concentration.—An alkaline reaction in the semen is associated with sterility (Webster, 1934-35; McKenzie and Berliner, 1937). Webster (1939*a*) found that distinctly acid ejaculates were of high fertility and that as the degree of acidity decreased towards the neutral point so did the fertility. Webster (1937) gave the average *p*H of fertile rams' semen as 6·85, of rams that failed at insemination as 7·72, and that of infertile rams as 8·23. According to McKenzie and Berliner, normal ejaculates with a sperm concentration higher than about 1 million per mm.^{3} were usually acid, sometimes as low as 5·9 and never as high as 7·3. In rams that were almost sterile the *p*H was often as high as 8·4 to 8·6. Comstock and Brady (1937) gave the *p*H of normal semen as 6·9 and of abnormal semen as over 7·0. Terrill (1937) observed that ejaculates giving an acid reaction to litmus were definitely superior to those giving an alkaline reaction. Very similar observations have been made by the writer. Although Gunn and co-workers (1942), using the electrical stimulation method, found that freshly collected semen very rarely showed any tendency to even a slight acid reaction to litmus, the writer, however, has commonly obtained acid ejaculates with this method.

Longevity.—Gunn (1936), who stored semen samples at 40° C. in a normally saturated atmosphere in tubes with cotton wool plugs and of such a size that only a comparatively small surface was exposed to the air, found that the average longevity of all semen samples was about 12 days. In a well-collected ejaculate the duration of motility should be about 10 to 21 days. Increased longevity of spermatozoa was obtained by injecting rams with pituitary extracts, anterior pituitary-like hormone of human pregnancy urine, acetylcholine and pilocarpine. A longevity of 64 days was obtained with sperm from a ram previously injected with fresh wether pituitary glands. Gunn and co-workers (1942) have evolved a method (graphical and mathematical) of expressing "effective motility" and the figure so obtained is called the "activity number." Since semen of high initial motility but of short longevity may have an activity number approximating that of semen of initial low motility but of very great longevity, the days of longevity are indicated in each case, thus: 1145/16 and 1194/56, the first figure in each case representing the activity number and the second the maximum days longevity. Increased longevity was observed in semen showing incipient degeneration (as much as 54 days) and in semen of rams recovering from degeneration (as much as 62 days).

Morphology.—The following classification of abnormal spermatozoa has been used in the ram (McKenzie and Phillips, 1934; McKenzie and Berliner, 1937): (1) Tailless; (2) coiled tails; (3) tapering heads; (4) broken at necks; (5) small and large heads; (6) enlarged middle piece; (7) middle piece bead; (8) filiform middle piece; (9) double heads; (10) double tails; (11) cytoplasmic extrusion at base of head. (*See* Fig. 4.) Tailless and coiled tail forms have been commonly noted by several workers. In the writer's experience, tailless forms are the most common abnormal

sperm, followed by coiled tails and broken necks ; the incidence of tapering heads and enlarged middle pieces is about the same ; other head abnormalities and filiform middle pieces are rare. According to Terrill the common types of abnormal spermatozoa include coiled tails, tailless heads, broken middle pieces or tails, and tapering heads. Other abnormal types found more rarely include enlarged middle pieces, middle piece beads, protoplasmic drops and odd sized heads. Gunn and co-workers have observed the following abnormal forms in the ram : heads separated from tails, and heads broken at the junction with their necks (found in cases of early acute degenerative changes), pear-shaped heads, diminutive heads, double heads, double tails, kinked tails, thickened tails and necks, truncated tails, and deformed heads and tails.

Abnormal spermatozoa in the earliest ejaculation of the breeding season or following periods of breeding inactivity, from satisfactory breeding rams, are mostly tailless or with coiled tails (McKenzie and Phillips). This is regarded as a physiological condition rather than pathological. Spermatozoa are continually being produced in the testes and are moving through the tract, so if they are not emitted they must, after a time, undergo degeneration and resorption. Immediately the older degenerating spermatozoa are cleared out, the subsequent ejaculates contain spermatozoa recently matured and moving up the tract, resulting in low counts of abnormal spermatozoa for satisfactory rams. It is therefore advisable to avoid the first one or two services following a rest period when collecting semen for a fertility test. McKenzie and Berliner also noted that the first ejaculates during the off season usually contained a higher proportion of abnormal spermatozoa than later ejaculates. However, using the electrical method for stimulating ejaculation, Gunn and co-workers (1936, 1942) at no time found an increase in the abnormal forms after a period of rest. The greater stimulation of the male genital tract with the electrical method, compared with natural coitus, resulting in a greater expulsion of sperm, may be responsible for this difference.

In rams of affected fertility, the chief head abnormality observed was tapering heads (McKenzie and Phillips). Twenty or more of these per 1000 spermatozoa have been observed in rams of very low fertility. In most cases of affected fertility abnormalities involving the middle piece or body are evident. Unsatisfactory rams all had high counts of tailless and coiled tail spermatozoa. In the semen of " good " rams the maximum number of tailless spermatozoa was 36 per 1000 ; coiled tails might number 96 ; in " fair " rams this number might increase to 240. It was concluded that rams with more than 140 abnormal spermatozoa per 1000 are probably of reduced fertility, provided the semen has not been collected immediately after a prolonged rest period. McKenzie and Berliner noted that one ram with a count of less than 50 abnormal spermatozoa per 1000 settled all his ewes in the first heat period. In another ram throughout September and the first half of October, the number of spermatozoa per mm.^{3} increased from 100,000 to 1,380,000 and the number of abnormal spermatozoa decreased from 848 to 150 per 1000, but none of the ewes inseminated with his semen during this period conceived. However, from October 13, all the ewes inseminated with his semen conceived and the number of abnormal spermatozoa fell below 150 per 1000 to as low as 27.

These authors also found that the relative number of abnormal spermatozoa increased in two seasons ; there was a short period in March and April with a small increase in the level of abnormal sperm, and a very pronounced rise in July, August, and September. There was, however, a distinct difference between these two seasons, for in April the concentration of sperm of most rams is high and in August and September the concentration and total number of sperm may fall to a very low level. There were marked individual differences between rams with regard to the extent of decrease in number and quality of spermatozoa produced during these periods.

McKenzie and Berliner state that it seems most reasonable to believe that fertility depends on the numerical proportion of abnormal spermatozoa present in any one ejaculate. Three hundred abnormal spermatozoa per thousand, reached by some rams in March and April, mean 750 million in an ejaculate containing 2500 million spermatozoa, leaving 1750 million normal spermatozoa. But if 300 abnormal spermatozoa per thousand were noted in August, unless the total number of spermatozoa in this particular ejaculate were known, the situation would appear no more serious than in April. In some rams the number of spermatozoa dropped to an extremely low level at this period. If 300 abnormal spermatozoa per thousand occurred in an ejaculate containing 70 million sperm, only 49 million healthy spermatozoa would remain capable of fertilising ova. These workers remark that this number becomes significant in view of the experiments of Kuznecov (1934), showing that at least 50 million spermatozoa per dose were necessary in cervical insemination, and those of Milovanov (1934) showing that eight to ten times that number were necessary for vaginal or natural insemination.

One important point raised by McKenzie and Berliner is whether or not the morphologically

normal spermatozoa in an ejaculate high in abnormal sperm retain their fertilising capacity or are affected in some undetectable way.

Green (1940*a*) using aceto-carmine stain and dark field illumination, observed a small, hyaline, vesicular structure contained in the membrane at the anterior border of some cells. The number of the vesicles present in fresh or stored samples varied between rams, and among samples from a given ram. The number of vesicles decreased with aging and with frequent ejaculation. No cell with an abnormal head was found with a vesicle, but tail pathology did not alter the percentage of cells exhibiting the vesicle. There was some evidence of a larger percentage of vesiculated cells reaching the infundibulum of the ewe in a given time. Green states that, because of physical differences, the anterior vesiculated region may play a more or less dynamic role in phenomena such as osmotic changes, permeability, or possible mechanical or enzymatic action associated with fertilisation. Samples having a larger percentage of vesicles are more desirable than other samples, because of the correlations found with certain physiological conditions of the sperm.

Gunn and co-workers (1942) describe certain types of cells, found only in abnormal semen and believed to be derived from the germinal epithelium of the testis. These are : (1) large, well-stained, multi-nucleated cells ; (2) similar but smaller, mono-nuclear cells ; (3) small, round, non-nucleated cells ; (4) large, poorly staining (degenerate) cells, or large cells often without nuclei but having numerous deep red granules which obscure the structure of the cell ; and (5) cells of various sizes and often boat-shaped, usually with irregular edges, some with deeply-stained nuclei, some without. The multi-nucleated cells (giant cells of Lagerlöf), together with cells of type (4), have been seen in the rete testis, in the epididymis, and in the ejaculated semen, and are termed " young germinal epithelial cells." According to Cunningham and Hopkirk (1935) they occur in advanced stages of testicular degeneration.

Boat-shaped cells have only been seen in the lower parts of the epididymis, in the ampullae, and in the semen. They are believed to be derived from other types of cells by attrition within the epididymis and vas deferens. Frequently they occur in the semen of rams that have recovered from previous, often marked, seminal degeneration, but whose semen shows no other abnormality. They are termed " old germinal epithelial cells."

These types of cells are considered to be of considerable diagnostic value, for young germinal epithelial cells have always been found associated with active degenerative changes in the semen, and old germinal epithelial cells with old-standing or recovered pre-existing cases of seminal degeneration.

Effect of Method of Collection.—Brady and Gildow (1939) compared the following methods of collection, (1) from vagina of forced mated ewe, (2) with artificial vagina at 40° F., and (3) by electrical stimulation. The artificial vagina gave the highest concentration and motility, and electrical stimulation the lowest concentration and the largest volume, the volume from the ewe being smallest. The results indicate that the artificial vagina provides the most useful method ; electrical stimulation has a definite use where semen is being collected from several rams in succession, or from rams that will not or cannot serve either a ewe or a dummy.

Using fresh and stored (24 hours) semen from Rambouillet rams, Terrill (1940) obtained 61% of pregnancies with semen collected from the vagina of the ewe, 52% from electrical stimulation of ejaculation, and 42% from the artificial vagina. The average volume was greatest from electrical stimulation and concentration was greatest from the artificial vagina.

General.—The seasonal changes in spermatogenetic activity observed by McKenzie and Berliner (1937) indicate on the whole a general association between certain semen characteristics ; high quality semen shows in general larger volume, higher concentration and total number of spermatozoa and smaller absolute and relative numbers of abnormal spermatozoa, and low quality semen the reverse. Also, in experiments on the frequency of copulation, reduction in concentration and total number of spermatozoa coincide with smaller ejaculates. However, the volume of the ejaculate may vary independently of the quantity of the spermatozoa, *e.g.* semen collected from all the Shropshires during June, July and August, and from two Shropshires during most of the year, and from one Hampshire in June was of normal volume but of low concentration. The early changes in the semen characteristics can be studied in certain conditions, such as heat degeneration.

Terrill (1937) noted that increases in volume, concentration, degree of motility, estimated percentage of motile spermatozoa, duration of viability, and proportion of normal spermatozoa were all associated. Duration of capacity for progressive motility was related to the duration of the capacity for motility. The index of reproductive capacity (the total number of normal spermatozoa produced in a 30-minute trial) was determined from the number of ejaculates per trial, volume of the ejaculate,

concentration of spermatozoa, and percentage of normal spermatozoa. The association of each of these 4 variables with the index, independent of the accompanying respective variation of the other 3 variables, was measured by third order partial correlation coefficients. The coefficients were significant for the number of ejaculations (0·76), volume of the ejaculate (0·68), and concentration of spermatozoa (0·75), but not significant for the percentage of normal spermatozoa. The association of factors not used in its calculation, such as degree of motility and duration of viability, with the index, indicated that the quality of the spermatozoa varied directly with the number produced.

There is an inverse relationship in the ram, as in the bull, between the concentration of spermatozoa and the *p*H of the semen, the greater the concentration, the more acid being the reaction. Comstock (1939) and Comstock and Green (1939) found a high correlation between the rate of glycolysis of fresh sperm and persistence of motility during storage. Comstock (1939) regarded the measurement of glycolysis as the best criterion for predicting the potential motility of fresh sperm. It is recommended that glycolysis rate per unit volume of semen, rather than per unit sperm number, be used, since it is weighted by metabolic activity as well as by density of sperm, both of which are associated with viability. Since the rate of glycolysis is sometimes higher in samples of high percentage of abnormal spermatozoa than in samples low in abnormals, both determinations need to be made and the two criteria used to supplement each other (for there may be many factors affecting sperm fertility that are completely independent of motility) for partial prediction of sperm fertility, and as a measure of sperm quality, especially in a comparison of different males or methods of storage. Green (1940*a*) found a positive correlation between the number of vesicles in the sperm heads and the rate of glycolysis.

Terrill (1938) compared the breeding capacity of Rambouillet ram lambs born in April with that of yearling and mature Rambouillet rams, to determine the probable extent to which these ram lambs might be depended upon for breeding. The ram lambs had sufficient mating desire and copulative ability for use in breeding. Services were obtained from 81% of the lambs compared with 93% of the older rams. Of the 21 rams tested, 18 could reasonably have been expected to give satisfactory breeding results and the other 3 would probably have sired offspring. These three ram lambs had a lower concentration and motility of spermatozoa than normal, and two in addition had 22% to 25% abnormal spermatozoa.

At the time of the first trial the ages of the ram lambs varied from 238 to 270 days with a mean of 249 days. The older ram lambs (average 258 days) produced a larger quantity of more actively motile spermatozoa in a given time than the younger ram lambs (average 241 days), although semen from the latter group contained a lower proportion of abnormal spermatozoa. Comparing ram lambs with older rams, the most definite age difference was in the greater production of spermatozoa by older rams. Rams 2 years and older were superior to younger rams. Comparison of the sperm production of sires and their sons gave a correlation of 0·40, which is on the border of significance. Arapov *et al.* (1935) concluded that 6 to 7 months' old ram lambs could be used for breeding, provided they received adequate feeding and were not overworked.

On the basis of their extensive study McKenzie and Berliner concluded that in estimating reproductive capacity in the ram it is important to consider the breed or strain, the season of the year, desire to mate, number of matings just prior to sampling, sperm concentration, total sperm number, proportion of abnormal spermatozoa, and volume of semen. A fecundity test which is based on morphological appearance of spermatozoa alone neglects important factors. A high count of abnormal sperm during the hot season should not be taken as a picture of the sperm producing capacity during the breeding season. After a long rest two or more ejaculates should be examined for an assay of fecundity. If it is planned to alter the breeding season of ewes by injection of sex hormones, then only such rams as do not lose their fecundity during that season of the year can be used as studs.

Fertility.—It is not possible to lay down hard and fast criteria for sperm characters in relation to fertility in the ram. The appropriate number of spermatozoa required for fertility has been indicated by Russian workers, but this number will probably vary with their morphological character and their functional activity. The determination of the *p*H of semen gives a measurement of general value in relation to fertility.

Terrill (1938) compared tests made immediately after breeding on 2 groups of rams, (*a*) those from which 95% or more (average 97·8%) became pregnant, and (*b*) those from which less than 95% (average 87·7%) became pregnant—the lowest ram in this group settled 84% of his ewes. The difference in fertility in the 2 groups was not great, neither was the difference in their semen. On the average the semen of the rams in group (*a*) was slightly superior in every respect. Terrill believed that the measurement of breeding capacity by semen tests would have value in providing more adequate criteria for selection of high fertility in the male.

Gunn and co-workers (1942) found no sharp line of distinction between highly fertile and sterile semen but rather a gradation through intermediate degrees of fertility from one to the other. As a rough working basis they suggested the following subdivision of semen types, according to their likely fertility under otherwise ideal conditions.

% Abnormal Spermatozoa.	Probable Fertility %.
0·1	80-100
1	60
10	45
30	20
>50	0

Semen showing signs of recovery from seminal degeneration, though still with marked abnormalities, is more fertile than similar semen which is not changing or which is in a state of progressive degeneration. Likewise semen showing signs of progressive degeneration, especially if it contains young germinal epithelial cells, is less fertile than similar semen which is stationary, or in a stage of recovery.

The association of seminal degeneration following acute fever with reduced fertility is demonstrated in the case of a ram whose fertility at the beginning of a service period was 78%. An intercurrent laminitis with a temperature as high as 107·6° F. caused rest from service for a week. When service commenced again the next week, fertility was less than 20%, at which it remained for 3 weeks, and for the subsequent 3 weeks it rose only to 45%.

Gunn and his colleagues have evolved a method for predicting seasonal fertility in rams from data on pasture conditions and atmospheric temperatures. This forecast is based on and for Australian conditions and may not be wholly applicable elsewhere. From meteorological data alone they consider it possible to predict, with some degree of accuracy, the type of semen likely to be possessed by entirely pasture-fed rams at any given time for a particular district. When rams receive supplementary feeding their semen is likely to be affected only by seasonal heat.

Their method consists of plotting for the locality the average daily maximum temperature and " useful " rainfall throughout the year, and from these data deciding the period of high quality semen produced and subsequently high fertility at mating. They regard periods during which the maximum temperature does not rise more than a degree or two above 90° F. as being not unfavourable for semen production, and since marked seminal changes do not occur rapidly as the result of seasonal heat, the dates of the seminal change, whether for better or for worse, are advanced 4 weeks in districts that experience extreme heat and 2 weeks in districts of less heat. Since the effect of improved diet on spermatogenesis in the ram is not evinced until 2 months after the beginning of such feeding, the date of the likely return of semen to normal due to dietetic causes is advanced 2 months. They estimate the onset of seminal degeneration caused by natural diet deficiencies as likely to be 6 months after the last " useful " rains. In some districts low temperatures, restricting growth of natural pastures, would have to be considered. They point out that anticipations of seminal degeneration based on diet deficiencies in natural pastures will not hold good if the rams receive any green pick or vitamin A supplement. They anticipate that the period of high fertility would fall during the period of predicted high quality semen and about a month before it. This month of the period of regeneration is included because they have found that semen of rams recovering from seminal degeneration, though as yet containing a fairly high proportion of degenerated spermatozoa, is highly fertile. There appears to be a good relation between the predicted fertility and the fertility of rams as observed by breeders in Australia.

Milovanov (1938) devised the following formula for analysing the relation between the quality of the injected sperm and the percentage of fertilisation :—

$$F = f \sqrt[p]{R} \,.\, \sqrt[m]{n}.$$

Where F = % of fertilisation
R = resistance of the sperm (see p. 96)
n = number of sperm in the dose of semen
f = coefficient of fertilisation, which depends on a number of factors apart from R and n.
m and p = indices of power, depending chiefly on the species.

The constant m is fairly well established (for sheep m = 5). The values of the coefficients f and p were worked out from a previous method (1937). For a given value of the root p, f should be constant

for different values of n, R, and F. The value of f approached a constant with a root of the fifth power. From Milovanov's experiments, the relationship between the number of injected sperm and their resistance on the one hand, and the percentage of fertilisation on the other, is expressed by the equation :—

$$F = 0{\cdot}24\sqrt[5]{nR}.$$

This formula for the inter-relation between fertility and dosage and quality of sperm also permitted the determination of the number of sperm required for attaining a high degree of fertility.

Transforming the formula $F = f\sqrt[5]{nR}$ we get

$$n = \left(\frac{F}{f\sqrt[5]{R}}\right)^5 = \frac{1}{R} \cdot \left(\frac{F}{f}\right)^5.$$

Thus, if the desired fertility (F) and the coefficient of fertilisation (f) are constant, then the necessary number of sperm in the dose is inversely proportional to resistance. Ordinarily 5% to 7% of re-insemination and 90% of fertilisations are considered good indices under Russian conditions ; taking $F = 90$ and $f = 0{\cdot}25$ we get :—

$$n = \frac{1}{R} \cdot \left(\frac{90}{0{\cdot}25}\right)^5 = \frac{1}{R} \cdot 6{\cdot}10^{12} \text{ (approx.)}$$

The calculation, from this formula, of the number of sperm in the dose in relation to resistance of the sperm in vaginal artificial insemination of sheep with gelatinised sperm is shown in the following table :—

Resistance after Dilution (R).	Inverse value of Resistance $\frac{1}{R}$.	No. of sperm per dose.	Possible No. Inseminations from this ejaculate.
1000	10^{-3}	6×10^{9}	1
2000	5×10^{-4}	3×10^{9}	2
5000	2×10^{-4}	$1{\cdot}2\times10^{9}$	5
10000	10^{-4}	6×10^{8}	11
15000	6×10^{-5} (approx.)	$3{\cdot}6\times10^{8}$	19
20000	5×10^{-5}	3×10^{8}	23
25000	4×10^{-5}	$2{\cdot}4\times10^{8}$	29
30000	$3{\cdot}3\times10^{-5}$	2×10^{8}	35
40000	$2{\cdot}5\times10^{-5}$	$1{\cdot}5\times10^{8}$	46
80000	$1{\cdot}2\times10^{-5}$ (approx.)	$7{\cdot}5\times10^{7}$	93

Instead of using the standard number of sperm (500 million) for vaginal insemination the number is varied in accordance with the quality of the sperm, taking a dose of 1200 million sperm with a resistance of 5000, and decreasing to 600, 300, 150, and even 75 million for sperm with higher resistance. Sperm with a resistance below 5000 should not be used, as it calls for doses of several milliards of spermatozoa. The number of possible inseminations with gelatinised ram sperm rises in direct proportion to resistance, and with a sufficiently high resistance may reach figures which do not fall short of those needed in cervical insemination. Provided the sperm is of high quality, it is possible with vaginal insemination to inseminate a large number of females with the sperm of a single ram. Present methods of feeding rams make it possible to attain with comparative ease a resistance of up to 30,000 or 40,000 (even 60,000), provided there is a sufficient preparatory period (not less than 2 months), during which the animals receive animal fodder, especially milk and blood meal (also bone meal).

To maintain a high resistance of the sperm after dilution, it is necessary to maintain the proper temperature conditions for dilution and to use a good quality diluent.

There was a direct relationship between the percentage of fertilisation in ewes and activity after

dilution and average resistance. Milovanov (1938), however, stated that there was no direct relation between the activity of the sperm and the coefficient of fertilisation, *f*, but that evidently variation in fertilisation at different activities was determined by variation in resistance.

The Semen of the Stallion

The ejaculate, which has a whitish, opaque appearance, usually consists of three fractions ejaculated in sequence. The first fraction is watery and contains few or no sperm ; the second fraction is thin and watery and contains the sperm ; the third fraction, which is viscous, comes mainly from the seminal vesicles and Cowper's glands.

Volume.—According to Lambert and McKenzie (1940) the volume of the whole ejaculate varies from 40 to 320 ml., the most common volume being from 75 to 150 ml. Day (1940*b*) gives data for 66 ejaculates from 4 Thoroughbreds and 15 ejaculates from one halfbred pony. The average total volume from the Thoroughbreds and the pony respectively was about 72 ml. (range 28 to 170 ml.) and 127 ml. (range 46 to 202 ml.). Davis and Cole (1939) using a two-year-old Belgian stallion, from which collections were made usually twice weekly, obtained a maximum of 170 gm. (approximately 6 ozs.) 49 days after collections were started.

There is considerable variation in the amount of the different parts of the ejaculate, particularly the thick viscous fraction. According to Lambert and McKenzie, the first fraction consists of from 5 to 10 ml. and the second from 25 to 75 ml. These two fractions are apparently grouped by Day into what he terms the " sperm serum." The average volume of the sperm serum for the Thoroughbred and pony is about 44 ml. (range 12 to 116 ml.) and 85 ml. (range 44 to 147 ml.) respectively.

Lambert and McKenzie state that the third fraction, if present at all, may be the most voluminous, constituting 44% to 71% of the total volume of the ejaculate. Davis and Cole observed variations of from no gelatinous material to nearly one-third of the total ejaculate. Day found that the average volume of the third fraction (vesicular fluid) for the Thoroughbreds and pony respectively was about 20 ml. (range 0 to 106 ml.) and 42 ml. (range 0 to 143 ml.). One stallion never produced any of this fraction. Frequency of collection was not the deciding factor as to quantity of this fraction (Davis and Cole). According to Lambert and McKenzie jacks do not produce much of the viscous fraction, except in old age, and stallions ordinarily do not produce it in the autumn. This viscous portion soon liquifies on standing.

Salzman (1940) obtained a mean volume of 54 ml. from 230 ejaculates from 17 normal fertile asses, mainly of the Hamadan breed.

Number of Spermatozoa.—Lambert and McKenzie give the range in sperm concentration as from 30 thousand to 800 thousand per mm.^{3}, with 60 thousand as the most common concentration. From Day's data, the concentrations of spermatozoa per mm^3. in sperm serum after separation from the vesicular fluid were for the Thoroughbreds and the pony about 110 thousand (range 20 to 233 thousand) and about 127 thousand (range 91 to 224 thousand) respectively. Salzman found an average concentration of 270 thousand per mm.^{3}

Davis and Cole (1939) noted that the range in number for a single collection was from a few hundred thousand to a maximum of 4500 million. This variation was apparently not related to the volume of the ejaculate. Day found that the average total number of spermatozoa (in millions) for the Thoroughbreds and the pony was about 5460 (range 340 to 27,028) and about 10,390 (5192-16,898).

Motility.—The motility for Day's Thoroughbreds and pony was from 60% to 100%. On the whole the motility was consistently good for these stallions. Salzman noted a mean activity of 0·8 in his investigations.

Morphology.—Voloskov (1935, 1936), using McKenzie and Phillips' classification, has established the following abnormal types in the sperm of the stallion : (1) pathological changes of the head—isolated heads, deformed heads, micro- and macrocephaly, abnormal situation of the head in relation to the tail, polycephaly ; (2) pathological changes of the tail—twisting of the middle part, abnormal curving of the flagellum, inclusions in different regions of the tail, the presence of the protoplasmic globule, filiform tail, absence of flagellum, double tail, acephaly. In Askania Nova the following classification is used (Milovanov, 1936)—giant, dwarf, tail deformity, head deformity, polycephaly, polyuria, acephaly, anuria and others.

According to Voloskov the percentage of deformed spermatozoa normally varies from 25% to 30%, and there exists a complete parallelism between the percentage of pathological forms and the percentage of foaling. Stallions with over 70% of normal spermatozoa fertilised 60% to 89% of their mares and those with 48% to 52% normal spermatozoa only 40% to 53% of mares. Voloskov

concluded that the morphological examination of sperm represents a valuable method of judging the breeding efficiency of a stallion, and that evaluation of sperm by motility and density may be misleading if the number of abnormal spermatozoa is high. Day noted very few abnormal spermatozoa in his 5 fertile stallions—and he concluded that relatively small numbers of abnormal spermatozoa have no bearing on fertility. One stallion had, however, from 35% to 45% of spermatozoa with a bead in the tail and 4% to 6% of double-headed sperm. Salzman noted only 5% of pathological types. According to Voloskov, husbandry has a distinct influence on the production of abnormal spermatozoa ; sexual over-activity, inadequate feeding, and lack of exercise lead to a reduction of the percentage of normal spermatozoa.

Savage, Williams, and Fowler (1930) measured the head lengths of sperm in 51 samples. The curves obtained were unimodal and their coefficients of variation ranged from 4 to 6 or slightly less. An unsound sire is indicated, they consider, by a coefficient in excess of 6, and animals which give skew curves may be regarded with extreme suspicion, since such curves are not obtained from normal animals.

General.—Day states that one collection of sperm may be sufficient to pronounce a stallion of good fertility, but several collections should be made before condemning a horse as of low fertility. He examined 2 completely sterile horses and in both of them the spermatozoa were dead ; in other horses of low fertility it was usually found that both the density and volume were very low, the density being about 10 to 15 million per ml. and the volume 10 to 25 ml.

The Semen of the Boar

A valuable and detailed study of the reproductive organs and semen of the boar has been made by McKenzie, Miller and Bauguess (1938) from which this account, unless otherwise stated, is taken.

The semen of normal boars is greyish to milky white in colour depending on the concentration of spermatozoa ; the higher the sperm concentration the whiter the semen. Fresh semen has no odour unless contaminated with urine or the contents of the preputial pouch. This pouch contains decomposing urine and cellular debris, which have a disagreeable odour—the characteristic sexual odour of the boar. About 60% to 75% of the whole semen is liquid and slightly viscous, and has a specific gravity of 1·01 to 1·02. Normal whole semen contains, in addition to the liquid portion, lumps of gelatin-like material resembling tapioca. In freshly ejaculated semen this material appears as chains of platelets, 3 to 5 mm. in diameter, with their flat surfaces attached, but these gelatinous bodies absorb liquid on standing, become greatly enlarged and settle to the bottom into a solid mass of jelly-like material. After cooling for 24 hours or more, they may take up the bulk of the liquid in the semen and comprise 50% to 75% of the total weight. This mass has an opaque grey colour, with a specific gravity of 1·03 to 1·04. Spermatozoa are present in the gelatinous material, apparently being trapped and held by it after ejaculation.

Some gelatinous material is found throughout the period of ejaculation, but it varies in appearance and quantity with the stage of ejaculation. In the first few minutes it is somewhat discoloured, perhaps by urine, and lacks the characteristic tapioca lumps ; instead it is a more uniform mass with the consistency of a thick lubricant. The largest amount of the typical tapioca material appears with or immediately following the high sperm-containing fractions. Near the end of the sperm-containing fractions the ejaculate is frequently all gelatinous material. In the interval between high sperm peaks, or if only one occurs, following it, a thin clear fluid appears, containing very few if any spermatozoa and having a specific gravity of 1·01 or less.

Volume.—The volume of the ejaculate normally varies from 125 to 500 ml., 200 ml. being the most common. Milovanov (1936) gives the volume as from 150 to 400 ml. There is no direct relation between live weight and semen volume.

The seminal vesicles, Cowper's glands, prostate and urethral glands, and the epididymis fluid contribute respectively 15% to 20%, 10% to 25%, 55% to 70%, and 2% to 5% of the semen volume. The liquid-gelatinous ratio is, on the whole, fairly constant, averaging about 70% liquid for the entire ejaculate.

Number of Spermatozoa.—The number of spermatozoa per mm.^{3} ranges from 25,000 to 1 million, the most common concentration being 100,000. (Milovanov (1936) gives the range as from 100,000 to 500,000 per mm.^{3}). The number per ejaculate ranges from 0·27 to more than 30×10^{10}. Eight collections from 3 boars gave an average total number of more than $8{\cdot}5 \times 10^{10}$ sperm per ejaculate (average sperm concentration of 305,000 per mm.^{3}). Five other boars gave a lower sperm concentration and total number of sperm per ejaculate ($1{\cdot}91$ to $2{\cdot}98 \times 10^{10}$), probably mainly due to the greater number

and frequency of ejaculations in these boars. According to Rodolfo (1934*a*) the average number of spermatozoa per ejaculate for a number of boars was $7{\cdot}83 \times 10^{10}$.

Because of the long and convoluted horns of the uterus of the sow, large quantities of semen are needed. Rodin and Lipatov (1935) recommended 100 to 150 ml. of semen diluted $\times 4$, depending on the size of the sow. McKenzie, Miller and Bauguess (1938) found that 50 ml. of semen free from gelatinous particles was sufficient. Lambert and McKenzie (1940) stated that volumes of 50 to 100 ml. of undiluted semen seem sufficient if the sperm concentration is reasonably high.

Morphology.—The classification of McKenzie and Phillips (1934) was used by McKenzie, Miller, and Bauguess. Coiled tails, middle piece beads, and enlarged middle pieces, appearing in that order, were by far the most common types of abnormal spermatozoa. Spermatozoa with a cytoplasmic cap completely and uniformly surrounding the head appeared in considerable numbers after extreme sexual activity and were interpreted as immature forms.

Rodolfo (1934*c*) described 3 types of spermatozoa, (1) those with no protoplasmic drop, (2) those with protoplasmic drop on neck, and (3) those with drop towards middle of tail. These types were believed to represent successive stages in sperm development. Spermatozoa in the proximal end of the epididymis were of type 2, and were transformed into type 3, and finally into type 1, which was predominant in semen.

Phillips (1935) concluded that the sperm morphology of a boar could be taken as an indication of his fertility. Semen of fertile boars had not more than 20% of abnormal forms. From 20% to 50% abnormalities occurred in semen of boars which produced small litters, dead or mummified foetuses, or from boars which failed to settle their sows. According to Milovanov (1936) normal fertile boars may have up to 30% of abnormal forms.

Hydrogen-ion Concentration.—The *p*H of normal whole semen ranges from 7·3 to 7·9.

General.—Rodin (1934), who collected the ejaculates (18 in number) in glass vials, changed every 15 seconds, distinguished 3 phases in ejaculation : (1) lasting 0·5 to 1·5 minutes and consisting of about 20 ml. urine-contaminated liquid containing few sperm ; (2) lasting 1·5 minutes and consisting of over 100 ml. semen containing some 34 billion sperm—the highest sperm concentration occurred in the first fractions of this phase, and sperm from these fractions retained their motility much longer than in the low concentration fractions (also noted by McKenzie *et al.*) ; (3) lasting 2·5 to 5 minutes, in which 164 ml. containing 2·2 billion sperm were ejaculated.

McKenzie, Miller, and Bauguess state that an ejaculation can be divided into three or five phases, depending on whether one or two sperm waves occur in the ejaculation. The first or pre-sperm phase, which lasts 1 to 5 minutes, consists of slightly urine-coloured, semi-solid material, contains no sperm and comprises 5% to 20% of the ejaculate. The second or sperm-containing phase, which lasts 2 to 5 minntes, consists of a milky white liquid and some gelatin- or tapioca-like material, contains most of the sperm, and comprises 30% to 50% of the ejaculate. The third or post-sperm phase lasts 3 to 8 minutes, consists of a thin, watery liquid with more or less gelatinous material, contains few sperm, and comprises 40% to 60% of the total volume. When there are two waves of ejaculation, the second and third phases are repeated, but the sperm concentration and volume are much lower than in the first wave. The second wave is more pronounced in some boars than in others, while in some animals it is entirely absent ; it tends to disappear with more frequent ejaculations.

Usually the rate of ejaculation increases up to the 3rd minute, reaches a peak in the 3rd, 4th, and 5th minutes and decreases thereafter for 2 or 3 minutes, to be followed by a second rise near the end of the ejaculation. There is thus a high initial peak, or wave, and a second lower peak, and coincident with these peaks in the rate of ejaculation are the peaks of sperm concentration.

The duration of ejaculation varies greatly in different boars, and there is no apparent relation between it and frequency of ejaculation. The duration ranged from 3 to 6 minutes (average 4·5 minutes) in one boar, to 11 to 25 minutes (average 16 minutes) in another. There is no direct relation between the duration of ejaculation and semen volume, nor between total number of sperm and semen volume. Rodolfo (1934*a*) also noted a great variation in volume and total number of spermatozoa, but no correlation between them.

In fractionated collections of semen the initial or pre-sperm fraction usually has a high *p*H, probably partly due to contamination with urine, which in the boar has a *p*H of 8·4 to 9·0. High sperm-containing fractions have a lower *p*H, and the intermediate and post-sperm fractions have a high *p*H but not as high as the pre-sperm fractions.

The initial part of the ejaculate probably serves the double purpose of cleansing the urethra of urine and debris, and acting as a lubricant to facilitate the entrance of the penis into the female tract. This material is usually discharged, in normal coitus, before the penis enters the vulva.

The chief function of the great volume of fluid ejaculated with and immediately following the sperm fraction is apparently to wash the sperm well into the uterus. The cervix of the sow does not evaginate ; the length of the vaginal and cervical tract combined is no greater than the depth to which the penis enters : ejaculation proper does not begin until the penis is inserted to its greatest depth ; hence there seems little doubt that the boar normally deposits his semen directly into the cervix.

The presence of materials from the seminal vesicles and Cowper's glands in the post-sperm fraction and the capacity of such substances for forming a rubbery, gelatinous mass, indicate that they may serve to seal the cervix, thus preventing the loss of semen. This vaginal or cervical plug is not, however, essential to fertilisation.

The Semen of the Dog

Lambert and McKenzie give the following data for the semen of the dog : volume of ejaculate 2 to 19 ml. (most common volume 7·0 ml.) ; sperm concentration per mm.^{3} 1 to 9 millions. Freiberg (1935) stated that the semen consists of 3 fractions ; the fluid from the glands of Littré, 1 to 2 ml. ; the fraction containing sperm, 2 to 4 ml. ; and the secretions of the prostate, up to 30 ml. Alifanov (1933-34) made observations on 30 dogs, dividing them into 3 groups, (*a*) those which attempted mating immediately, (*b*) those which did so only after several hours' interval, and (*c*) those which completely ignored the female. For semen collected with the artificial vagina, animals of group (*a*) yielded 2·5 ml. of dense sperm with an average concentration of 189 million sperm per 1 ml. ; in group (*b*) the volume was 2 ml. and the concentration 74 million ; and in (*c*) 1·5 ml. and 14 million. Observations on 2 dogs demonstrated that sperm were produced continuously, and the normal amount was restored at intervals of 24 to 72 hours.

The Semen of the Fox

Starkov (1933-34) gives the following particulars about the semen of the fox : average volume 1·5 ml., the range being 0·1 to 4·5 ml. ; average concentration 66 million sperm per ml., with a minimum of 34 and a maximum of 120 million ; *p*H 6·2 to 6·4. The number of ejaculates obtained from one male during the breeding season varied from 4 to 16 ; one male gave 18 but the 16th and later ejaculates were small, with a low concentration, and feebly motile or defective sperm. The day on which the first ejaculation was induced varied in different individuals, so that apparently the onset of spermatogenesis was not simultaneous in all males. Starkov collected semen by mechanical manipulation, but Lambert and McKenzie state that this method has not proved successful. With the electric stimulation method from 0·5 to 1 ml. was obtained from a fox at each collection.

The Semen of the Rabbit

Lambert and McKenzie give the following particulars about rabbit semen : volume 0·4 to 6·5 ml. (most common volume 0·7 ml.) ; sperm concentration per mm.^{3} 100 thousand to 2 million (most common 700 thousand) ; *p*H 6·8 to 7·5. Macirone and Walton (1938) noted that with an increase in the total volume of ejaculate there is an increase in the total number of sperm, but the relationship is not linear. Individual variation in the concentration of sperm is considerable. With successive matings the number of sperm diminishes rapidly, and as the density is not much affected the diminution is due to reduction in the volume of ejaculate. Seasonal variation was not marked ; there was a slight tendency for the number to be highest in the early part of the year and lowest in August and September when the animal is moulting. Walton (1933) reported that satisfactory insemination results were obtained with 0·5 ml. of semen which had been diluted 32 times. Lambert and McKenzie gave the recommended volume for the doe as from 0·25 to 1·0 ml.

The Semen of the Fowl and Turkey

Volume.—According to Lambert and McKenzie, the fowl produces from 0·1 to 1·5 ml. per ejaculate, the most common volume being 0·6 ml. For the turkey the volume is from 0·1 to 0·8 ml., the most common volume being 0·3 ml. Hutt (1929) noted a considerable variation in the amount of the ejaculate of different cocks, and of the same individuals at different times.

Number of Spermatozoa.—Lambert and McKenzie give the range in the number of spermatozoa per mm.^{3} for the fowl as from 50 thousand to 6 million. Hutt found a range from under 19 thousand

to over 8 million, with an average of just over 4 million for 36 counts. For individual cocks the average count ranged from over 800 thousand to over 6 million, and within this range the density of sperm bore no relation to fertility. Burrows and Quinn (1938) stated that insemination with 0·1 ml. of semen once a week should result in fertility in 80% to 95% of the eggs. Munro (1938) stated that probably about 100×10^6 sperm must be placed in the vagina of the hen before fertility will remain at a normally high level for a period of 10 days.

Hydrogen-ion Concentration.—The *p*H of the semen of the cock ranges from 6·3 to 7·8 (Lambert and McKenzie).

General.—Hutt noted that the density of the sperm was independent of the amount of the ejaculate. Comparing high- and low-fecundity strains of White Leghorns, Jones and Lamoreux (1942) found that the former produced a greater volume of semen at 12 weeks of age and this superiority was maintained at greater ages. Since there was little difference in the concentration of spermatozoa between these two strains, it was concluded that the average volume of semen produced by a group of several males was a reliable measure of the relative numbers of spermatozoa produced.

Centrifuging fowl semen allows about 75% of its volume to be removed as supernatant fluid free from spermatozoa (Munro, 1938). Since the fowl lacks accessory glands found in mammals, it might be suspected that differences between cockerels in the production of accessory fluid would be somewhat less than those between individual mammals (Jones and Lamoreux).

CHAPTER 3. FACTORS AFFECTING SEMEN PRODUCTION

Knowledge of the physiological basis of male reproduction is essential for the full understanding of male fertility. Changes and variations in the ejaculates of males must be referable to the organs that produce the component parts of the semen. In this chapter are reviewed briefly the more salient features of male reproduction from this viewpoint, paying particular attention to the larger animals and laying, where possible, most stress on functional conditions in the male genital organs, which may be reflected in the semen, and on factors both internal and external which affect them.

Complete reproductive performance in the male comprises essentially the production of semen containing normal spermatozoa, together with the full mating reaction. Male reproductive functions are controlled to a large extent by internal factors, mainly the secretions of the pituitary and the testis. They are also, however, influenced by external factors such as climate and nutrition. The internal control is based on (1) the production of gonadotropic hormone by the pituitary, and the response of both seminiferous epithelium and the interstitial tissue of the testis to it; and (2) the production of testis hormone by the testis, under the influence of gonadotropic hormone, and the response of the accessory glands and ducts to it (Moore, 1939). There must also be proper functioning of the erectile and ejaculatory mechanisms and the desire to mate, all of which are probably due to some form of neuro-humoral mechanism (Stone, 1939*a*).

Testis

The primary organ in the male reproductive system is the testis, which has two distinct functions: (1) the elaboration of spermatozoa, and (2) the production of an internal secretion.

Spermatogenesis.—In many animals spermatogenesis is cyclical, occurring usually once a year; but in domesticated animals it is more or less continuous throughout reproductive life (*see* seasonal variations, p. 39). There may be, however, a considerable variation in the stages of spermatogenesis in different seminiferous tubules, and in some tubules spermatogenesis may be completely absent (Lagerlöf, 1934). The occurrence of normal and degenerated tubules and of tubules with partial degeneration explains the simultaneous appearance of healthy and abnormal spermatozoa and of other testicular cells in the one ejaculate (Lane-Roberts *et al.*, 1939). This variation in spermatogenetic activity is apparently physiological, though the condition of the tubules is also markedly affected by pathological processes. Various factors, such as seasonal influences and dietetic deficiencies, can cause reversible degeneration of the tubules. During the processes of degeneration and of regeneration, some tubules will be affected to a greater extent than others, and spermatogenetic activity will exhibit less tubular variation at the period of maximum reproductive activity than at other times. Variation in spermatogenetic activity will have a corresponding effect on the spermatozoa themselves.

Phillips and Andrews (1936) first noted marked development of the germinal epithelium at

84 days of age in the ram, at 142 days in the bull, and at 84 days in the boar. Spermatozoa first appeared at 147 days in the ram, 224 days in the bull, and 147 days in the boar. In a study of sexual development in small and large types of the Poland-China breed of pigs (Phillips and Zeller, 1943), spermatozoa were first observed at 20 weeks and were present in all pigs at 25 weeks. Rapid development of the germinal epithelium began after approximately 17·5 weeks. The seminiferous tubules began to increase in size between 10 and 15 weeks, and grew rapidly up to about 30 weeks, after which age there appeared to be little increase in size. The testes began to grow rapidly between 17·5 weeks and 20 weeks, and growth continued at a fairly constant rate up to 40 weeks.

Edwards (1940) noted in the rabbit a highly significant positive relationship between the weight of testicular substance and the number of spermatozoa which it produced ; following unilateral castration there was no hypertrophy of spermatogenetic function, nor of weight of testis.

From a study of the semen production of White Leghorn males from two strains selected for high and low fecundity, Jones and Lamoreux (1942) noted that although males of the high fecundity strain were significantly heavier than those of the low fecundity strain, the average weights of the testes in the two strains were not significantly different. Since, however, the testes in the high fecundity strain produced larger amounts of comparable semen than those of similar size in the low fecundity strain, they must have been more active or more efficient in the production of spermatozoa. Histological studies confirmed the difference in activity in the testes of the males of the two strains, for at 8 to 24 weeks of age there were more metaphase plates and more spermatozoa present in the seminiferous tubules of the testes of males of the high fecundity strain. A larger proportion of the tubules were active in the high fecundity males after 20 weeks of age. This study suggests that semen production in the male, and egg production in the female, are expressions of comparable genotypes for high and low fecundity.

Endocrine Function.—The internal secretion of the testis controls the development and activity of the passages providing egress for the spermatozoa and a fluid vehicle for their transport (Moore, 1939). The functional activities of the epididymis, the deferent ducts, the seminal vesicles, prostate, and penis depend on this secretion. Sex desire is influenced by it. The accessory gland and duct functions are very quickly affected by a failure of the secretion as, for example, after castration in the rat, which causes cytological changes in the cells of the seminal vesicles within 48 hours. The functions concerned with mating, which depend on a neuro-humoral mechanism rather than on a purely hormonal one, are much less affected by withdrawal of the secretion.

Cytological and physiological tests are used to determine the effect of testis hormone on the accessory glands. There are different threshold levels of effectiveness upon the different end organs (Moore and Gallagher, 1929). The order of sensitivity, as determined by the androgenic effect of testis tissue extract, is : the spermatozoon motility test (most sensitive) ; the prostate cytology test ; seminal vesicle cytology ; Cowper's gland ; ductus deferens ; the electric ejaculation test, least sensitive (Moore and Gallagher, 1930). The spermatozoon motility test is a physiological indicator of hormone activity. In the guinea-pig, rat, and mouse, capacity to show motility is retained for 54 to 70 days, 30 days, and 60 days respectively, provided one testis is present (Benoit, 1925 ; Moore, 1928). When both testes are removed motility is lost in 20 to 23 days in the guinea-pig, 18 days in the rat, and 14 days in the mouse. The testis hormone is thus responsible for prolonging the life of spermatozoa within the epididymis. It has also been determined that the secretion of the testis hormone is a continuous process and that the body does not store the secretions. The degree to which epididymal secretion, and indirectly testis hormone, may be responsible for poor motility of spermatozoa in ejaculates, and perhaps to some extent for variation in different ejaculates, remains to be decided. Another functional test for the testis hormone is the *p*H of the seminal vesicle fluid (Lanz, 1929). The normal *p*H was 6·34 but 40 days after castration it was 7·38.

There is a general relationship between the production of germ cells and hormone secretion. Species, such as the ground squirrel (Wells, 1935), which have a seasonal spermatogenetic cycle, also have a seasonal hormonal cycle, although there may not be an exact coincidence in time between the two cycles. It also appears that species which do not have definite restricted breeding cycles may, nevertheless, undergo seasonal changes in their reproductive organs and hence in their semen. The fact that both the spermatogenetic and endocrine functions of the testis are more or less similarly affected would seem to indicate a common control, which is probably by the pituitary. Both spermatogenetic and endocrine functions of the testis may, however, vary to some extent independently of each other as, for example, in cryptorchidism in which the secretion of hormone continues but spermatogenetic function is absent. It seems, however, that cryptorchid testes may secrete smaller amounts of hormone than normal ones (*see* Moore, 1939).

In the dog, castration caused prostatic atrophy in 7 to 23 days. The restoration of prostatic secretion following injections of testosterone propionate, 5 to 25 mg. daily, followed a smooth curve to form a plateau, which was interrupted occasionally by a prolonged elevation with return to the established level. The prostate once having been reconstructed, the dosage could be greatly reduced without causing a decrease in secretion. The secretion was unaffected by removal of the thyroid and parathyroids. Hyperthyroidism caused a secretory depression interrupted with returns to normal levels (Huggins, Masina, Eichelberger, and Wharton, 1939).

From the studies of Phillips and Andrews (1936), Andrews (1940), and Tyrrell, Andrews, and Zelle (1942) it appears that the mere mechanical presence of the testis plays no part in maintaining the scrotum in a physiologically normal condition, and that the internal secretion of the testis is the sole regulator of scrotal activity. Selye (1943), however, concluded that both mechanical distension of the scrotum and the stimulating effect of testoid compounds are required for the development of a normal scrotal pouch.

It has been noted that testis hormones have an inhibiting effect on spermatogenesis. In the ram, Gunn and co-workers (1942) observed that the injection of 10 capon units of proprietary preparations of male sex hormone on 3 successive days produced an immediate rise in semen quality followed by a marked fall, including seminal degeneration.

Accessory Organs

The secretory activity of the accessory reproductive organs of the male is dependent, as has been pointed out, on testicular hormone and it can also be depressed by certain nutritional states, as described later (*see* p. 34). Any alteration in accessory gland function will have a corresponding effect on the seminal fluid. The spermatozoa motility test shows that a delicate relationship exists between spermatozoa and the function of the epididymis. It is possible that physico-chemical tests may aid in locating functional alterations or pathological disturbances in the accessory organs.

Epididymis.—Apart from being a passage for the spermatozoa, the epididymis acts as a storehouse for spermatozoa and also provides suitable conditions for the maturation of spermatozoa. It is a secretory organ and therefore contributes, to some extent, to the seminal fluid. In the bull the epididymis is probably the main storehouse, for the first few ejaculates after vasectomy contain very few, if any, spermatozoa (personal observations). Yet spermatozoa can be obtained by massage of the ampullae of the vasa deferentia in the bull so a certain number of sperm may be stored here, though massage of the ampullae may of course stimulate contraction further back in the genital tract. In the ram it appears that the vas deferens may contain a considerable number of spermatozoa (Gunn, 1936). In the rabbit, on ejaculation, the vas deferens practically empties and some of the sperm come from the cauda epididymis (Macirone and Walton, 1938).

Evidence on the maturation of spermatozoa during their passage through the long ducts of the epididymis is based on (1) the differences in fertility with spermatozoa from different parts of the tract, (2) morphological changes which spermatozoa are considered to undergo during "ripening," and (3) the protective film or lipoid capsule which, Redenz (1924) believes, the spermatozoa acquire during their passage.

Spermatozoa from the testes are usually not capable of fertilising ova (Moore, 1939). Immature spermatozoa from the testicular end of the epididymis of the guinea-pig gave only 33% fertility compared with 68% for mature spermatozoa from the proximal end (Young, 1931). In the bull, rat, dog, guinea-pig, and rabbit spermatozoa are very low in motility when they leave the testes and this power gradually develops as they pass through the epididymis (Young, 1929*a*). For the sperm of the cock Munro (1935) demonstrated that the grade of motility is lowest in the testes, greater in the epididymis, and highest in the vas deferens. The degree of fertility was parallel to the motility. The sperm of the cock thus undergoes a process of maturation after formation, but it does not mature completely in the epididymis. This is probably related to the very small size of the epididymis in the fowl, and presumably the spermatozoa pass through it quickly to the long coiled vas in which they spend the greater part of the time before ejaculation.

The two views commonly held on the maturation of spermatozoa are (1) that it is due to the secretions of the tract, and (2) that it is an aging process in the spermatozoa themselves, the spermatozoa being endowed with a certain survival capacity (Young, 1929, 1931). It is not unlikely that both views may be at least partly correct, for it has been clearly shown that spermatozoa do undergo an aging process (Moore, 1928; Young, 1929*b*) and that the epididymal secretion is essential for prolonging the life of spermatozoa. The essential point seems to be the actual passage through the

epididymis, for a considerable decrease has been demonstrated in the time of transport in rams subjected to frequent artificial ejaculation without any alteration occurring in the longevity of the spermatozoa *in vitro* (Gunn, 1936). The ability of the male to maintain high quality semen after many ejaculations is further evidence for the rapidity of maturation ; Hammond (1921) showed that in the rabbit coitus as frequent as 39 times in 8 hours did not affect fertilisation.

Although, therefore, a rapid passage through the epididymis does not apparently have any detrimental effect on spermatozoa, too long a stay in the epididymis is harmful. When spermatozoa were confined to the epididymis for longer periods by ligature, the over-ripe spermatozoa only gave 25% fertility (Young, 1931). In the guinea-pig and rat there is a gradual falling off in the vigour of movement of the spermatozoa both in number and intensity, and Moore concluded that it appears to be a case of the spermatozoa growing old and gradually losing their vibratile powers. If not ejaculated, spermatozoa undergo dissolution. As spermatogenesis in the larger animals is a continuous process, the spermatozoa in the tail of the epididymis or in the ejaculate must represent a mixed sample of all ages, and the preponderance of younger or older types will depend on the frequency of ejaculation.

Redenz (1924) considered that mammalian sperm retain their normal functional ability as long as the lipoid capsule is intact. During passage through the epididymis, in addition to becoming surrounded by a colloidal capsule rich in lipoids, sperm acquire other properties, namely a high negative charge, and the capacity for anaerobic glycolysis (Milovanov, 1933). The formation of this capsule is considered to lead to a great increase in the resistance of sperm to external conditions, namely acidity and temperature.

Spermatozoa in the epididymis retain their motility much longer than their fertilising capacity. The retention of motility and fertilising capacity in the rabbit are 60 and 30 days respectively (Hammond and Asdell, 1926), in the guinea-pig 60-70 days and 20-35 days (Moore, 1928 ; Young, 1929*b*), and in the rat 42 and 21 days (Moore, 1928 ; White, 1933). Kirillov and Morozov (1936) isolated the left epididymis and removed the right epididymis and testis of a bull. Five days after the operation the bull yielded 6 ml. of semen containing 300 million spermatozoa showing an activity of 0·5 ; 32 days later the corresponding figures were 2 ml., 100 million spermatozoa and 0·7 activity. Thereafter the percentage of spermatozoa showing progressive movement decreased ; oscillatory movement was observed for over 2 months, but eventually all spermatozoa became immotile.

The period of motility given by Moore (1928) is the maximum period for which even the feeblest motility persisted. Fairly good motility persisted for 41 days in the guinea-pig and for 18 days in the rat. Considerable individual variation in both these animals was noted. The period for which fertilising capacity is retained is more nearly equal to that of good motility than to the longest period of motility. These data for the length of fertilising capacity in the epididymis of the different species may indicate the period for which fertilising capacity may eventually be retained *in vitro*.

Epididymal fluid is generally considered to be the most favourable medium for maintaining the viability of spermatozoa. The epididymis does not permit an excessive accumulation of metabolic products or exhaustion of nutritive material. According to Milovanov, the low content of the epididymal fluid in Cl ions and electrolytes generally and its acid reaction favour the transition of the sperm in the tail of the epididymis into the resting stage, which is further aided by the lower temperature of the scrotum. In the presence of organic acids, spermatozoa are said to become very sensitive to a fall in temperature and may pass into the resting stage at 30° C.

Redenz considered, according to Milovanov, that the acid reaction of the epididymis is due to the acid products of sperm metabolism, but Lanz (1929) believed that this acid reaction is due to the secretory activity of the epididymis itself, which is influenced by the hormonal secretion of the testes, since (1) removal of both testes created an alkaline reaction in the epididymis, and (2) an acid reaction is present in the epididymis of young animals containing no spermatozoa.

Passage of Spermatozoa through the Epididymis.—Spermatozoa when first cast off by the Sertoli cells are crowded in a thin aqueous medium which is most likely a kind of transudate of the tissue fluid surrounding the tubules (Cowdry, 1934). Pressure within the testis, supplied partly by the continuous production of spermatozoa and partly by the contraction of the dartos and cremaster muscles, which has been observed by Gunn (1936) in the ram after copulation and artificial production of ejaculation, probably causes oozing of the spermatozoa through the vasa efferentia. The increased blood pressure within the comparatively inexpansible tunica albuginea may help to express the spermatozoa from the tubules and rete testis into the vasa efferentia. According to Marshall and Halnan (1932) the ejaculatory contraction begins in the walls of the vasa efferentia, passes to the

epididymis, and thence along the vas deferens. During electrical stimulation Gunn has noted that the bared epididymis decreases in volume and its coiled tubes contract, giving it a wrinkled or knotted appearance. The cilia in the vasa efferentia, whose beat is in general towards the epididymis, probably help to push on the sperm and fluid (Zawisch-Ossenitz, 1933).

Phillips and McKenzie (1934) observed that after scrotal insulation, which caused heat degeneration of the testis, the time required for spermatozoa to pass through the genital passages of a frequently ejaculating ram was on the average 8·8 days (range 4 to 13 days), the average number of services during this period being 8·2 (range 4 to 12). These findings were based on the assumption that the increased abnormal forms occurred in the testis during spermatogenesis. Gunn (1936) studied the rate of passage of spermatozoa in the ram by injecting Indian ink into the rete of the testis (thus ensuring that the ink passed the whole length of the semen tubules from the vasa efferentia to the exterior), and by a combination of ink injection and scrotal insulation. He found that in 6 frequently ejaculating rams (electrical stimulation, 3 times in 5 days or 7 times in 9 days), the first appearance of Indian ink and abnormal spermatozoa was in 5 days (longest 6 days). Gunn considered that these results, obtained by two methods, substantiated Phillips and McKenzie's assumption that after scrotal insulation the increase in abnormal spermatozoa in frequently copulating rams occurs during spermatogenesis in the testis. Since the rate of passage of spermatozoa through the epididymis of rams induced to ejaculate frequently by electrical stimulation (5 days) is more rapid than in those allowed frequent service (8·8 days), the number of spermatozoa ejaculated must be greater with the former method.

For a study of the effect of early changes in the testis on the sperm of the ram, the electrical stimulation method of causing ejaculation would seem to be the method of choice. The results of Gunn and co-workers (1942) suggest that a single ejaculation in a ram which is on the threshold of seminal degeneration is sufficient to increase the rapidity of the development or the degree of degeneration. Deficiency in the number of spermatozoa is more likely to be revealed by the artificial stimulation method than by natural coitus. In sexually rested rams, Gunn found that the time taken for injected ink to appear in the ejaculate was 11 days.

In the rabbit the passage of spermatozoa through the epididymis may be accomplished in 4-7 days (Edwards, 1940). The rapid passage of sperm through the epididymis of the ram (5-6 days) did not adversely affect the longevity *in vitro* of the sperm (Gunn).

Other Accessory Glands.—Knowledge of the secretions of the other accessory glands is still incomplete. Their physical and chemical properties differ between the glands and from those of the epididymis (*see* p. 60).

It is sometimes considered that the accessory secretions have a favourable influence on spermatozoa, but Redenz was unable to establish either a stimulation of the spermatozoa or a prolongation of their life. It was considered by Milovanov (1934) that the high content of electrolytes (particularly NaCl) and the alkaline reaction of accessory secretions induce motility by terminating the anabiotic condition of the sperm in the epididymis, but at the same time cause, by changing the medium, a disintegration of the lipoid capsule similar to that observed when sperm are treated by a solution of NaCl, and in this way shorten the duration of life of the spermatozoa. There are several instances of the unsuitability of seminal fluid for the preservation of sperm. From a study of the change in the *p*H of bull semen after one hour's incubation it would seem that seminal fluid is not on the whole a good medium for the maintenance of high motility (Anderson, unpublished data).

In the boar it appears that removal of the contributions of the seminal vesicles and Cowper's glands from semen reduces the number of abnormal sperm and increases the duration of sperm motility. Sperm in semen from boars without seminal vesicles and Cowper's glands remained motile longer than sperm in normal semen from the same boars, and this was probably due to increase in sperm density. Similarly, sperm in the high sperm fractions from normal boars, which were probably most nearly free from seminal vesicle and Cowper's gland contributions, remained motile longest. The presence of seminal vesicle and Cowper's gland secretions is not essential to fertility in the boar (McKenzie *et al.*, 1938).

The volume is probably of more importance in the stallion and boar than in the bull and ram, since the two former ejaculate large quantities of semen which probably has a physiological function in aiding the progress of sperm in the female genital tract. Walton (1933) stated that it seems likely that the function of the accessory secretions is mainly mechanical. The seminal fluid gives bulk to the ejaculate, rendering the peristaltic contractions of the urethra more effective in forcing the sperm to the exterior.

Pituitary

The dependence of gametogenetic function upon the pituitary gland has been clearly established in many species. The possible influence of other endocrine glands is much less direct and is probably exerted more through their effect on body function in general.

There is very little clear evidence of the effect of administering gonadotropic hormones on spermatogenesis in the larger animals, but some reports indicate possible beneficial effects. McKenzie and Berliner (1936) reported a definite increase in sperm numbers in a young beef bull of extremely low fertility after treatment with 18,000 R.U. Antuitrin S (a human pregnancy urine preparation). In 18 days after the end of the treatment the bull had reverted to his former low level of fertility. (The right testis on removal showed marked tubular degeneration.) Pregnant mare serum (8000 to 10,000 R.U.) was more potent and a marked improvement followed its administration, with a definite improvement of spermatogenesis in the remaining testis. In no case, however, did conception follow mating. Bottomley, Folley, and Watson (1940) treated 3 sterile bulls, whose semen showed a deficiency of spermatozoa and absence of spermatozoa, with Antuitrin S. Two injections of 1000 R.U. each at 4 days' interval caused a marked improvement in concentration and motility of spermatozoa with successful breeding results. In man the most hopeful field for gonadotropic therapy is in cases of relatively slight deficiencies of spermatogenesis (Lane-Roberts *et al.*, 1939).

Skvorcov and co-workers (1934) noted a marked rise of sexual impulse in one bull after injection of 320 M.U. of prolan.

Gunn and co-workers (1942) injected 200 R.U. of the gonadotropic hormone of pregnant mares' serum into a ram in which seminal degeneration had occurred from feeding on mature pasture hay (probably due to inadequate vitamin A intake) ; the semen returned to normal in 2 months though the ram's body weight fell very considerably and it was very emaciated. Rams kept on sufficient diets were unaffected by the hormone. The cure of seminal degeneration due to dietary deficiencies in rams by gonadotropic hormone and by concentrates containing only small amounts of vitamins A and D, supports Turner's (1938) contention that vitamin and protein deficiencies adversely affect reproduction by inhibiting pituitary function.

Thyroid

Although the thyroid has not been found to have a direct effect on the testis, there is good evidence that an indirect effect is exerted as the organism finds itself in a state of metabolism in which many functions are somewhat impaired (Moore, 1939). Hart and Stieve (1921) observed that mice exposed to environmental temperatures of 32° to 40° C. showed progressive degeneration of the seminiferous epithelium and characteristic degenerative changes in the thyroid. Smelser (1937) found that hyperthyroidism in the rat depresses both sperm production and accessory gland function. He considered that this hypofunction of the male reproductive tract may be explained by an increase in the threshold of response to gonadotropic and male hormones. Thyroidectomy also causes a marked decrease in sperm production and a definite reduction in the weight of accessory reproductive organs of the male rat (Smelser, 1939), demonstrating a definite though not extreme depression of testicular function. The reduced weight of the accessory organs is regarded as indicating a reduction in testis hormone secretion. The decrease in both parts of testicular function is not explicable by a comparable drop in the gonadotropic potency of the pituitary. Thyroidectomy must decrease the output of gonadotropic hormone of the anterior pituitary.

Berliner and Warbritton (1937) found that, in rams, sperm production decreased after thyroidectomy and increased following administration of thyroxine. They concluded that in regions in which high temperatures prevail before and during onset of the breeding season, rams with a high range of thyroid activity are better able to reproduce than animals with thyroids of low activity. Low thyroid activity is believed to cause low sperm production in summer. It was suggested that the reaction of the thyroid to either external or internal stimulation might be regarded as a hereditary constitutional factor of breeds ; Shropshire rams for example, showed marked destruction of germinal epithelium and poor sperm production in summer and in a high temperature room during winter, while Hampshires were little affected. Rams with abnormal spermatozoa should be regarded with suspicion if they do not respond to thyroxine therapy. Thyroxine can prevent the summer decline or restore sperm production in the autumn. The observations of McKenzie and Berliner (1937) indicate a close relationship between the thyroid and testis in the ram. Low thyroid activity may be associated with both obesity and poor sperm production.

Thyroidectomy in the bull causes complete cessation of libido but has no effect on spermatogenesis or on the normality of the sperm (Petersen *et al.*, 1941). Normal activity and sexual behaviour are restored by the oral administration of 25 g. dessicated thyroid and by dinitrophenol and testosterone propionate. Following thyroid therapy in the bull, the writer has noted an improvement in the motility and *p*H, but there is no effect on the concentration of spermatozoa.

In man thyroid deficiency is often associated with sterility and loss of libido and potency. A decrease in the number of spermatozoa and an increase in abnormal forms is often found in cases in which thyroid function is deficient and these findings, together with the results of thyroid medication, suggest that a certain level of thyroid activity is essential for testicular efficiency (Lane-Roberts *et al.*, 1939).

Temperature

It has been clearly demonstrated in mammals that testis function requires a temperature lower than that of the body (*see* Moore, 1939 ; Phillips and McKenzie, 1934). Reproduction is probably affected by heat to a greater or lesser extent in males of all species. The effect of heat has been studied in the ram (Phillips and McKenzie, 1934 ; Gunn, 1936 ; McKenzie and Berliner, 1937 ; Gunn, Sanders and Granger, 1942) and in the bull (Lagerlöf, 1934). Heat applied locally to the scrotum, either experimentally, or by local inflammation, as well as an increase in body temperature due to summer or hot climates, or to exercise or febrile states, may affect not only spermatogenesis but also fully formed sperm. Much can be learnt from the experiments and observations on heat degeneration in the ram and bull, not only about the changes in the semen and their relation to testicular degeneration, but also about the actual sequence of the seminal changes. This is of considerable value for the study of variation in semen properties.

Thermo-regulatory Function of the Scrotum.—Temperature control of the testis is a function of the scrotum exercised by virtue of its exposed position, thin walls and its response to temperature (Crew, 1922 ; Moore, 1939). The response to temperature is accomplished through the tunica dartos muscle. Increase in temperature relaxes this muscle, thus removing the testis from the warmer body temperature, and a lowering of the external temperature causes contraction of this muscle. The testes are apparently less susceptible to a decrease than to an increase in temperature (Phillips and McKenzie). The tunica dartos is most sensitive to changes at temperatures approximating the normal scrotal temperature ; at temperatures of 6° to 24° C. there is constant adjustment of the scrotum, the tunica dartos contracting and relaxing as a result of very small decreases or increases in scrotal temperature. Phillips and Andrews (1936) found that the tunica dartos muscle first showed a marked reaction to temperature changes at from 63 to 84 days in the ram and at 105 days in the boar, while results in the bull were somewhat variable. Their experiments indicate that in the ram the tunica dartos is dependent upon a testicular hormone for the development and maintenance of its reactivity to temperature changes. Heat applied to the testis produces hyperaemia associated with oedema, and it is possible that the heat degeneration may be due to lack of oxygen and accumulation of CO_2 due to vascular stagnation (Moore, 1924 ; Barron, 1933).

Gunn and co-workers (1942) from their observations on the greater frequency and degree of dependence of the scrota in Merino, as compared with some other breeds of rams, and on the smaller size and number of sweat glands in their scrotal skin, concluded that, for local heat loss from the scrotum, Merino rams depend on radiation and conduction to a greater extent, and on evaporation to a less extent, than do most other breeds. They point out also that the presence of a woolly or hairy covering on the scrotum may be rather protective than harmful in hotter districts by preventing conduction of heat to the scrotum.

Body Temperature.—Since the testes are susceptible to a rise in body temperature, the means by which body temperature is regulated and the effectiveness with which this mechanism maintains body temperature in different species and in individuals of the same species, is of importance for male reproduction. Since the testes of some rams continue to function even at temperature ranges that are decidedly detrimental to the germinal tissue of other rams, McKenzie and Berliner (1937) consider it probable that superimposed on the thermo-regulatory function of the scrotum is the more complicated metabolic temperature-regulating mechanism of the body, involving the nervous and endocrine systems. Gunn and co-workers point out that the remarkable susceptibility of the ram to heat is doubtless related to his very poor ability to lose heat by sweating, for in hot weather vaporisation of water in the air passages is the main, and almost only, means of general heat loss.

The heat-regulatory system is more severely tested in cattle of European types than in Zebus. The effect of temperature on cattle has been investigated by several workers, notably by Rhoad

(1936, 1940), who found that external temperatures above 73° F. greatly increased the metabolism of European cattle and only slightly that of Zebu cattle. Very similar results have been recorded in South Africa (Bonsma, 1940), in Tanganyika (French, 1940) and in Kenya (Daubney, 1942). Bonsma has noted high body temperatures, associated with extremely high scrotal temperatures in beef bulls under hot environmental conditions. In addition to the effect that high temperature alone may have on testis function, the loss of energy caused by high temperatures may affect male reproduction adversely. A further complicating factor may be the low nutritional plane experienced under dry conditions and in drought years when the temperature is high.

The importance of such findings for tropical and semi-tropical countries is obvious. In Kenya, however, there is no evidence that atmospheric temperature conditions adversely affect reproductive functions in purebred and grade bulls to any appreciable extent. Daubney has shown that almost the whole of the areas in Kenya where stock raising is already established enjoy, as indicated by mean annual temperature below 65° F., a climate of the type that is regarded in other countries as eminently suited to the raising of cattle of European breeds. In some areas and in certain seasons temperature conditions may be more adverse. In Kenya it has been noted that apart from the actual temperature to which the animal is exposed, the period of exposure has a greater effect in grade than in Zebu cattle (personal observations). The intermittent nature of periods of high temperature and the low daily minimum temperature experienced in the highlands of Kenya probably offset, to a great extent, any adverse effect that high temperature may have on male reproduction.

Seminal Degeneration in the Bull

In the bull Lagerlöf (1934), by means of scrotal insulation, observed a direct relationship between changes in the semen picture and changes in the seminiferous epithelium. As in the ram, the longer the period of insulation the more marked were the changes in the number of sperm and the increase in abnormal types. The increase in the number of abnormal spermatozoa started about 11 days after the beginning of insulation, the insulation being carried out on alternate days and lasting for 96 hours. Motility was the first to be affected (5 to 9 days). There was an increase in " unripe " spermatozoa (*i.e.* spermatozoa with protoplasmic drops on the neck) about the same period as the increase in abnormal types.

Bonsma (1940) has made some interesting observations on Afrikaner and beef bulls in South Africa, and noted that bulls belonging to exotic beef breeds often have body temperatures of 106° F. or more, and extremely high scrotal temperatures (115° F. or more). It is highly probable that such temperatures adversely affect sperm production. The skin of the scrotum is said to be about twice as thick in Afrikaner bulls, namely, 0·4 cm. compared with 0·15 to 0·2 cm. in exotic bulls. When the temperature rises the scrotum of the Afrikaner is retracted and the skin becomes puckered. The testes are drawn against the perivisceral cavity so that their temperature does not rise above that of the body, which in Afrikaner bulls is seldom higher than 102° F.

A decrease in semen quality in the bull has been noted after anaplasmosis and foot and mouth disease and it would seem that such results are probably due to increased body temperature affecting spermatogenesis rather than to the actual disease process (personal observations). In one bull just recovered from foot and mouth disease the concentration of spermatozoa was normal, but the motility was poor (20%) and the *p*H was rather high (6·86); nine weeks later the motility was good (80%) and the *p*H lower (6·60), since when the bull has maintained good sperm production. Inflammation in one testicle may disturb spermatogenesis in the other through the rise in scrotal temperature and the disturbance in the heat-regulating function of the scrotum (Lagerlöf, 1934, 1936). If the affected testicle is not removed the high temperature remains for some time and after about a fortnight spermatozoa are very few or absent. Lagerlöf stated that if the affected testicle is removed immediately at the beginning of the disease it is generally possible to save the sound testicle. There is no reason for delaying unilateral castration since a testicle which has been subjected to acute inflammation will rarely produce fertile spermatozoa.

It has been observed in Kenya that under colder conditions bulls are less keen to serve, and produce poorer semen. Herman and Swanson (1941) mention a bull which was apparently affected by cold icy weather; the quality of the semen decreased, initial motility was often poor and survival time of good motility lessened. With the coming of warmer spring weather the semen became of much better quality. This was the only one of 10 bulls which showed this apparent seasonal variation in semen quality.

Seminal Degeneration in the Ram

In the rams studied by Phillips and McKenzie (1934) the body temperature averaged 39·8° C., and at room temperatures of 13° to 24° C. the scrotal temperature averaged 33·3° C. and the testicular temperature 34·9° C. Scrotal insulation increased the scrotal temperature to 36·4° C. (+3·1° C.) and the testicular temperature to 37° C. (+2·1° C.). With rams confined to a closed room the effect of high temperatures (with a probable high humidity) was almost the same as for scrotal insulation (McKenzie and Berliner, 1937). In these rams the scrotal temperature averaged 34·5° C. (*i.e.* 1·2° C. above normal), and the rise in body temperature was from 0·56° C. to 1·12° C. In general the longer the period of insulation the more marked was the degeneration of the germinal epithelium (Phillips and McKenzie). After 4 days a notable amount of degeneration was present and after periods longer than 2 weeks no spermatozoa were observed in the tubules. The spermatids and the secondary spermatocytes were the first to be affected, followed by the primary spermatocytes and the spermatogonia. Once degeneration was started, even by a brief application of heat, much of the germinal epithelium tended to break down before rebuilding set in. The probable delay in the return of the hyperaemic state to normal after removal of the heat may have been partly responsible for this effect. Gunn and co-workers (1942) observed that the changes in the semen of rams exposed to an artificial hot dry atmosphere were essentially similar to those in rams whose scrota had been insulated. The temperature and humidity conditions to which the rams were exposed were based on the high temperature and low humidity which occur in New South Wales (the actual temperatures and humidity which are shown graphically indicate approximately temperatures of about 70° to over 100° F., with considerable periods over 90° F. and relative humidity from about 40% to 60%).

Before discussing the effects of heat on the semen it must be recognised that the early detection of changes in the sperm will depend on the rate of passage of the spermatozoa through the epididymis (*see* p. 26), and that this is likely to be more rapid in rams induced to ejaculate with electrical stimulation than with natural coitus. With rams subjected to scrotal insulation or hot dry atmospheres, the changes tend to be so rapid that the special features of incipient degeneration are not always detected, the reason being that the collection of semen does not always coincide with the short period during which these characters are exhibited.

Number of Spermatozoa.—In Phillips and McKenzie's rams (scrotal insulation) an average of 20 days (range 13 to 25 days) elapsed from the beginning of scrotal insulation until the sperm supply was exhausted, and the average number of services during this period was 14 (range 9 to 18). In Gunn's frequently ejaculating rams there was a marked decrease in the number of sperm in 5 days and whole spermatozoa were completely absent from the ejaculates in from 8 to 11 days, indicating a rapid and complete expulsion of the entire contents of the epididymis. McKenzie and Berliner's rams showed an obvious decrease in the number of spermatozoa in 11 to 14 days after the start of exposure to higher temperature. Some rams showed a more rapid decrease in sperm number than others and this seemed to be related not only to the number of ejaculations, but also to the spermatogenetic level. The data of Gunn and co-workers also indicate apparently individual levels in spermatogenesis.

Motility.—Gunn (1936) noted that motility decreased 5 days after the beginning of insulation in frequently ejaculating rams, and in 8 days it was completely absent. In the artificial hot dry atmosphere the decrease in motility to 70% was related to the actual exposure period in the early stages, *e.g.* an initial motility of 70% was maintained for 4 to 7 days, with exposure periods of 92 to 47 hours, and for 10 days with 32 hours' exposure.

Longevity.—In frequently ejaculating rams there was a marked decrease in the period of longevity about 6 days after the beginning of insulation. In the graphical presentation of the effective motility of spermatozoa collected before and after exposure of rams to the artificial hot dry atmosphere, and stored at 4° C. (Gunn *et al.*) it appears that the period of maintenance of good motility (motility factor 70 and over) is reduced at 3 to 4 days after the start of exposure to a degree depending on the period of exposure.

p*H.*—An increase in alkalinity occurs in the semen of rams subjected to scrotal insulation and to a high temperature room (McKenzie and Berliner). The first increase in alkalinity was observed at 6 days in Gunn's rams (tested with litmus).

Morphology.—A marked change in the morphology of ejaculated spermatozoa was noted, the number and type of abnormal spermatozoa increasing considerably after scrotal insulation (Phillips and McKenzie). The first increase in the number of abnormal spermatozoa was noted in one ram 4 days after the beginning of insulation, and the average period for 5 rams was 8·8 days (8·2 services

during this period). The rams in which abnormal spermatozoa appeared first were those in which a scarcity of spermatozoa was first noted. With high body temperatures, abnormal spermatozoa appeared approximately at the same period as after scrotal insulation, the earliest being 6 days (McKenzie and Berliner). In frequently ejaculating rams the first appearance of spermatozoal abnormalities occurred 5 days after scrotal insulation (Gunn) and 4 to 5 days after exposure to a hot dry atmosphere (Gunn *et al.*). McKenzie and Berliner observed that the first abnormalities to occur consisted of tailless spermatozoa and spermatozoa with coiled tails. Later there was an increase in the number of spermatozoa with tapering heads, broken necks, and enlarged middle pieces. Gunn (1936) noted that abnormal spermatozoa resulting from raised scrotal temperature appeared in a definite sequence—spiral necks, coiled tails, broken tails, heads separated from tails, followed by disintegration of all spermatozoa. Round cells somewhat resembling spermatocytes appeared. Abnormalities other than coiled tails and tailless spermatozoa thus seem to indicate a severe and advanced stage of degeneration.

Clinical Changes in Testes.—After scrotal insulation Phillips and McKenzie noted a marked decrease in testis size. Gunn observed a rapid change from the normal, plump, rather solid organ to one which was small, soft, and flabby. In the artificial hot dry atmosphere, clinically obvious softening and decrease in the size of the testes invariably appeared a few days after the estimated time of derangement of spermatogenesis (Gunn *et al.*). When the degenerative process was rapidly progressive and the ram was caused to ejaculate frequently, the clinical phenomenon occurred earlier, which suggests that it is due to the emptying of the tubules of the testis and the failure of their replenishment, caused by the absence of spermatogenesis.

General.—The available data indicate that the various changes in the semen of the ram, the decrease in motility and longevity, the increase in the number of abnormal spermatozoa and in the *p*H, all appear at about much the same time after exposure to heat. Gunn observed that the reduction in concentration and total number of spermatozoa in the ejaculate occurred 4 days after the rise in abnormals. From the results of Gunn and co-workers there appears to be more individual variation in the time required for a marked decrease in the number of spermatozoa (per stimulation) than in the other characteristics. It might be expected that functional changes in the semen would occur before any others, but it may be that present methods are not sufficiently delicate to reveal them. McKenzie and Berliner's data indicate that a rise in *p*H precedes or accompanies an increase in abnormal spermatozoa. The period for which good motility is retained on storage is affected as early as 3 days after exposure to a hot dry atmosphere (Gunn *et al.*). The earliest appearance of other changes was at 4 days.

In the study of seasonal changes in the semen of rams (*see* p. 39), changes in sperm numbers, activity numbers, longevity and reaction to litmus were the earliest, the most sensitive and, in cooler districts, almost the only indication of a seasonal effect. Changes in sperm morphology tended to lag behind the other seminal characters in their time of development. In cooler districts in which degenerative changes were minimal, sperm morphology was not a valuable indication of seasonal effects ; in hotter districts, on the other hand, in which seasonal changes were more marked, sperm morphology was the most valuable criterion (Gunn *et al.*). In the initial stages of degeneration Gunn and co-workers observed that collection of semen by the electrical method was always more easily effected, in that fewer stimulations were required to obtain a suitable sample. The sperm numbers per stimulation, the activity numbers, and longevity were much increased. These effects were possibly due to the collection of a more perfect sample of semen, or to some special characters of the semen such as its reaction, or degree of admixture with accessory secretions, and were presumably produced on spermatozoa which were already fully formed at the start of the fever and were possibly due, partly at least, to an initial stimulating effect of the heat. The occurrence of morphological changes at this time depends on the severity of the cause and the rapidity of its action. Gunn and co-workers state that " If the cause is mild and acting slowly, all the spermatozoa may remain morphologically normal and the other characters alone then indicate incipient degeneration. On the other hand, if the cause is severe and acting rapidly, there may already be definite morphological signs of degeneration, concurrent with the other special characters typical of incipient seminal degeneration." They observed that exposure of rams to hot atmospheres resulted in a graded departure of the semen from the normal according to the degree of heat and duration of the exposure. A continuous period of exposure had a much more severe effect than intermittent exposure for the same total period, spread over a greater number of days.

Fly Strike.—Blowfly strike in sheep is accompanied by a rise in body temperature, and Gunn and co-workers have found that seminal degeneration in rams follows fly strike on any part of the

body. Severe and protracted natural strikes are followed by marked degeneration and semen may contain few if any normal spermatozoa. Fly strikes on the scrotum, even if moderately restricted in area, produce much more marked and persistent effects than strikes elsewhere. If natural strikes receive suitable early treatment the degree of seminal degeneration and its duration are decreased, but failure to treat and subsequent re-strikes produce the reverse effect. Treatment with glycerol boric acid dressings has no harmful effect on spermatogenesis, such as that observed with strong arsenical or phenol dipping fluids.

Mating.—It has been observed by Gunn and co-workers that the excitement and exercise associated with service lead to a definite rise in body temperature, and since they have observed that rams whose body temperatures are constantly raised 1° to 2° F. for long periods invariably suffer seminal degeneration from which they recover only when their temperatures return to a constant normal level, they consider that this temperature rise in rams associated with service may well be the cause of the seminal degeneration which they have noted after mating. Paddock service causes more severe seminal degeneration than does comparable hand service, and this difference is probably due to the greater number of services and the increased amount of exercise, with the consequent greater effect on body temperature (differences in nutrition may, however, be involved).

These results lend support to the practice of selecting ewes with teasers for presentation to a stud ram at hand service, rather than of mating singly or allowing the ram to do his own teasing, both of which would probably lead to greater seminal degeneration. They also support the practice of using one lot of rams for the first half of the joining period and another for the last part.

Transportation.—Gunn and co-workers mention the view of breeders that rams transported over long distances in humid trucks, particularly in hot weather, are seldom highly fertile after their arrival. They consider that driving rams long distances in hot weather, particularly in the heat of the day, is likely to cause seminal degeneration of a greater degree than would be the result from the climatic conditions alone.

Recovery from Seminal Degeneration.—There is considerable individual variation in the response to heat (Phillips and McKenzie ; McKenzie and Berliner ; Gunn *et al.*). Some rams are more resistant than others ; individual rams vary greatly in the time and degree of response to elevated temperatures ; and certain temperatures are critical for some rams while others are only affected by still higher temperatures. Some rams are capable of overcoming the adverse effects of critical temperatures sooner than others. After complete and long-standing seminal degeneration, caused by scrotal degeneration or by subjection to a hot, dry atmosphere, or after severe and extensive blowfly strikes or other causes of fever, recovery takes about 2 months, being longer in warm than in cooler weather (Gunn *et al.*). With less seminal degeneration, recovery is shorter, varying according to the degree of the initial degeneration. An interesting observation is the tendency of rams to recover from an acute seminal degeneration of short duration despite unfavourable seasonal conditions, as for example recovery from blowfly strikes in rams in hotter districts, while other rams show marked seasonal seminal degeneration.

Seminal changes rather similar to those seen in incipient degeneration, but with certain differences, have been observed in cases of early recovery from seminal degeneration. Sperm numbers per stimulation, longevity, activity number and volume of semen are greater and the reaction is essentially similar. There may, however, still be present a considerable percentage of abnormal spermatozoa.

Effect of Heat on Fully Formed Spermatozoa

In the guinea-pig the higher temperatures of the abdomen shorten the length of life and vigour of motility of spermatozoa within the epididymis (Moore, 1928 ; Heller, 1929). Knaus (1932) has shown that, in comparison with the duration of fertilising capacity of sperm in the scrotal epididymis, which is about 40 days in the rabbit, the duration of fertilising capacity in the cryptorchid isolated testis is about 7 days and in the cryptorchid epididymis about 4 days. Also in the ram it seems that fully formed spermatozoa are affected by a rise in temperature. The results of McKenzie and Berliner's experiments support this view, for the first appearance of abnormal spermatozoa after increase in temperature was not associated with a decrease in sperm concentration. The first were comparable to abnormal spermatozoa found in ejaculates after a prolonged sexual rest. Phillips and McKenzie, for example, noted a high abnormal count consisting mainly of tailless and coiled tail spermatozoa during the first few collections after a period of some months of sexual inactivity ; after a few services these degenerate spermatozoa were cleared out and normal spermatozoa were ejaculated. Gunn, however, found that the first artificially produced ejaculate following a considerable rest period

contains at the most only an insignificant number of abnormal forms (confirmed by Gunn *et al.*, 1942). He observed that in sexually rested rams ink injected into the rete required 11 days to appear in the ejaculated semen, while degenerative changes were first observed in 7 days. There was an increase in spermatozoa with coiled tails and longevity was reduced. Gunn believes that these effects were produced as a result of the increased temperature acting on spermatozoa which were already in the epididymis at the beginning of the scrotal insulation. It thus seems that heat can induce morphological changes in spermatozoa in the epididymis, as shown by the coiling of tails and separation of tails from heads. (*See also* p. 136.)

The shortest time required for the passage of spermatozoa through the epididymis is 4 to 5 days, so any alteration in sperm characteristics in a shorter time is presumably due to an effect on the fully formed spermatozoa in the epididymis, as for example the reduction, 3 days after exposure to a hot dry atmosphere, in the period for which good motility is retained on storage. Other seminal changes, such as decrease in initial motility and increase in *p*H, may be similar.

Light

It has been abundantly demonstrated in recent years that seasonal reproduction is conditioned, to a greater or less extent, by the duration, intensity, and wavelength of illumination (Bissonnette, 1933, 1936; Marshall, 1936). This does not, however, apply equally to all animals. The ground squirrel, for example, is not stimulated by light treatment (Wells, 1935). In birds and small animals the effect of light is exerted through the pituitary. The possibility that light may be associated with reproductive function in the larger animals must be kept in mind.

Light stimulates spermatogenesis in several species of birds, and Lamoreux (1941) has shown that it stimulates the production of semen in Leghorns.

Nutrition

A considerable amount of information is available about the nutritive factors required for normal male reproductive functions in laboratory animals. Much less is available for the larger animals, though this lack of knowledge is gradually being overcome, and in one or two instances the need for certain food constituents for male reproduction has been demonstrated.

Friedman and Turner (1939) concluded that with notably few exceptions the dietary requirements for reproduction, qualitatively and quantitatively, do not exceed those for maintaining mature animals in good health. The exceptions are vitamin E, vitamin A, and protein. Vitamin E is so widely distributed in nature that, for the present at least, vitamin E deficiency may be regarded as a laboratory curiosity. It is regarded as quite possible that protein deficiencies not readily detectable in the mature animal can produce lowered breeding performance. Vitamin A represents a problem of practical importance, for with moderate restriction of vitamin A intake, mature animals may suffer from reproductive disturbances without showing any obvious signs of ill health. It is probable, moreover, that the degree of vitamin A deficiency required to produce these disturbances can be reached in ordinary farming practice when the stock is fed low-grade hays for a period of several months. Yet Friedman and Turner state that even in the case of protein and vitamin A the requirements for successful reproduction have not been shown to be beyond those for normal growth. Apparently in dairy bulls a ration which provides for fairly normal growth to 3 years of age is satisfactory for normal reproductive performance (Jones *et al.*, 1942),.

It is therefore necessary to take a broad view of the feeding of males used for breeding and aim at providing an optimum ration. The ration should be well balanced and provide ample protein, carbohydrates, minerals, and vitamins. Some animal protein should be included in the ration. A supplementary mineral mixture may be necessary, its composition depending on local circumstances. Green food should be fed whenever possible. Green pasture, fresh green lucerne, and leafy vegetables will ensure a supply of carotene (also from carrots), ascorbic acid, and vitamin E. The first two substances are important in the bull, and probably in other animals, and vitamin E may also be so. Lack of green feed, and hence of vitamins, during prolonged dry season in the tropics or semi-tropics may have detrimental effects on males. When the breeding season is restricted, feeding on these lines should start some weeks before and continue during the breeding season.

Plane of Nutrition.—The effect of inanition on the male reproductive system has been reviewed recently by Mason (1939). He uses the term in its broadest sense to cover quantitative and qualitative inadequacies of diet which result in general under-nourishment of the organism other than those involving specific deficiencies of, for example, one or more vitamins. Inanition during prepubertal

life retards or arrests sexual development, delays puberty and suppresses spermatogenesis. The early proliferative activities of undifferentiated germ cells and spermatogonia are not significantly impaired, and it is only after extreme inanition that the spermatogonia are affected. There is a striking tendency for the testis to increase in weight under conditions of inanition which permit no increase in body weight.

In the adult the germinal epithelium offers much more resistance to inanition, and in the rat it is only after a loss of from 25% to 35% of body weight that sperm formation ceases. The spermatogonia are more or less unaffected but the spermatocytes are sloughed or undergo dissolution *in situ*. The germinal epithelium thus assumes a rather inactive and quiescent appearance resembling that of the prepubertal state. It is noteworthy that in the rat these changes can be completely repaired following return to an adequate diet after 1 to 3 months, depending on the severity and duration of the inanition.

Inanition may depress the production of testis hormone and cause atrophy of the prostate and seminal vesicles in the rat (Moore and Samuels, 1931). Sexual desire and reproductive ability are usually maintained during the early stages of inanition, but are gradually lost as the physical debility increases.

According to Phillips and Andrews (1936) progressive degeneration of seminiferous tubules occurs in boars kept on a low plane of nutrition. Comstock and Brady (1937) found that a low level of even a high quality diet results in the production of reduced quantities of semen, although still of high quality. A very similar observation has been made by Gunn and co-workers (1942). In rams receiving liver meal lick (high in vitamin A) the semen was still normal but the number of spermatozoa was reduced at the end of 5 months, whereas in rams receiving linseed cake seminal degeneration occurred in 3 months, the numbers of spermatozoa remaining high. There was a much greater loss of weight in the former rams than in the latter.

It is well known that male animals kept in high condition for shows are often of poor fertility. McKenzie and Phillips (1934) have found that rams in high condition may have many abnormal spermatozoa and hence be of poor fertility or even sterile. One ram which had just come off the show circuit had 75·9% of abnormal spermatozoa and did not settle any of 34 ewes. Within 5 weeks of shearing and a slight reduction in weight the count of abnormal spermatozoa dropped to 15·8%, but as he was once more fitted for the show ring his total count mounted gradually over a period of 4 months to 69·2% abnormalities. However, fattening does not always reduce the breeding capacity of a male (McKenzie and Berliner, 1937). Some animals gain in weight more easily than others and likewise lose their breeding capacity sooner than others. Both properties, it is considered, may be due to the same endocrine make-up regulating both the metabolic and gonadal processes.

Friedman and Turner (1939) point out that almost all nutritional deficiencies in animals are complicated by underfeeding. Food intake on the deficient ration is much below normal since any diet deficient in one or more ingredients is apparently less palatable to the animal than a well-balanced diet. Specific dietary deficiency may thus be complicated by energy deficiency.

According to Marshall and Hammond (1943) the best condition for breeding in males is a hard one, produced by sufficient exercise to work off a surplus of fat, but favouring the retention of nitrogenous substances and vitamins. Moreover, a rising condition is better for reproductive functions than a falling one.

Protein.—Cunningham and Hopkirk (1935) have produced sterility in male rats by (*a*) feeding protein in excess (65% to 85%) and (*b*) feeding protein biologically poor (large part of protein derived from maize and maize and gelatin). The deficiency of some of the essential amino-acids in maize, and maize and gelatin, is considered to be the cause of the sterility, and the hypothesis is advanced that for proper nutrition the testes require a liberal supply of these amino acids. This view is strengthened by later work (Cunningham *et al.*, 1937). With these high protein diets and diets in which the protein is derived from maize, a somewhat definite series of changes occurs both in the seminal fluid and in the testes, the abnormalities becoming progressively more marked the longer the rats are on the diet. The earliest change noted in seminal fluid is low motility. The motility decreases progressively, accompanied by decrease in the density of spermatozoa, until eventually spermatozoa are all non-motile or are absent. Morphological changes in the early stages include loose heads and turning back of the head on the midpiece. The next stage shows tails turned back on the midpieces and often masses of degenerating spermatozoa, frequently headless. At a later stage the head is turned back on the midpiece and the whole spermatozoon is circled by the tail; spermatids may be seen. In the final stage there is cell-sloughing with degenerated bundles of sperm tails and an almost complete absence of heads.

On analogy with the results obtained in rats it is suggested that the bull sterility, which occurs in the Taranaki and Waikato (New Zealand) districts where the protein content of pasture is very high (crude protein content of grass may be as high as 35% of the dry matter), may be due to the narrow nutritive ratio of the pastures. Sterility has been noted in boars which were fed rations probably higher in protein content than normal. Nordby and Bollen (1930) reported a case of temporary sterility in a boar fed on Canadian field pea screenings. Complete fertility was restored in 6 to 7 weeks after a change of diet to barley, oats, tankage, oil meal, alfalfa and sodium chloride. At the time when the diet was changed the *p*H of the semen was 7·61 and the motility poor. On the altered diet the *p*H fell to 7·35 and the motility improved.

Russian workers have found that the feeding of animal as distinct from vegetable protein increases the sperm production of bulls, rams, and boars (Smirnov-Ugrjumov, 1937 ; Lysov *et al.*, 1937 ; Hudjakov, 1938). It is possible that the difference between animal and vegetable protein may be due to lack of essential amino-acids in the latter. The experiments of Cunningham and Hopkirk and of Courrier and Raynaud (1932), who found that rats fed on a diet deficient in the amino-acid lysine have relatively poorly developed testicles, provide clear evidence of the importance of certain amino-acids for normal male reproductive function. When the protein in a diet is derived from vegetable sources the adequacy of the supply of all the necessary amino-acids will depend on the " complementary " action of the vegetable proteins.

Comparing rations for the bull containing (1) oats, (2) oats and concentrates, and (3) a ration including bone meal and skimmed milk, it was found that (2) caused an improvement in sperm quality, especially volume of ejaculate, concentration and resistance of spermatozoa, and (3) caused an even greater improvement (Smirnov-Ugrjumov). It is concluded that appropriate feeding, including feeds of animal origin, will make it possible to increase considerably the number of services and to improve the quality of the sperm, which will result in a better utilisation of valuable sires. The beneficial effect of protein was not manifested for 17 to 37 days (Smirnov-Ugrjumov and Laptev, 1938). Green grass without concentrates resulted in an immediate decrease in sexual activity and amount of ejaculate. Herman and Swanson (1941) stated that it is recognised by experienced dairy cattle breeders that feeding large amounts of silage is detrimental to fertility in the bull.

In the ram the following foods were effective in the following order in respect to volume and number of spermatozoa : millet, barley, sorghum, and oats. Activity was also raised by protein feeding (Popov and Okuličev, 1936). The sperm production of 50 Merino rams (4400 semen samples) was examined under different dietary regimes by Okuličev and Meščerjakova (1934). Four different grain rations, balanced to have the same protein content, were compared. It was found that oats and millet had a stimulating effect on sperm production, but rams fed barley showed a markedly greater endurance during intensive service, while rams with a high protein ration were much more eager and produced more sperm. The addition of calcium phosphate led to an increased sperm production, and of iodine to greater motility of the sperm. Milk and eggs had a favourable effect, making the ram more ardent and increasing sperm production. It is estimated that the production of 1 ml. of sperm requires on the average 80 g. of digestible protein (Okuličev, 1939). According to Tommê and Odinec (1940) the amount of energy expended for one mating is 126·8 Cal. or 54 g. of starch equivalent, which is only about 15% of the amount required for maintenance ; in calculating rations the amount of nutritive substances should be calculated per mating and not per cm.^{3} of sperm.

It has been found that a rich protein diet improves the quality of sperm in the stallion (Popov *et al.*, 1936). Eggs and bran are said to have a stimulating effect on the production of semen by the stallion (Polovcova and Nagaev, 1929). The addition of 75 grams daily of cattle brain to a diet of meat and groats in the dog is said to have a marked beneficial effect on sperm production and libido (Alifanov, 1935).

Vitamin A.—Deficiency of vitamin A in the mouse, rat, guinea-pig, and bull causes atrophy of the testes and degenerative changes in the seminiferous tubules. Degenerative changes usually precede outward manifestations of deficiency (*see* Mason, 1939). In the male rat a deficiency causes a rapid degeneration of the germinal epithelium with resultant loss of fertility (Mason, 1933). With marked deficiency the proliferation of basal cells is greatly reduced or suppressed, but complete removal of the germinal cells, as after vitamin E deficiency, has not been observed. The very degenerate testes respond rapidly to therapy, a characteristic which distinguishes vitamin A deficiency from vitamin E deficiency. In the rat, developing spermatozoa are plentiful by the 45th day, and usually by the 60th and invariably by the 90th day the testes are fairly normal. Fertility becomes re-established after the appearance of mature spermatozoa.

Hart and Guilbert (1937) found no spermatozoa in the testes of a bull that died of vitamin A

deficiency; but another bull, which was so badly affected that it became permanently blind and had frequent convulsions, successfully impregnated 6 cows after vitamin A feeding had removed all evidence of the deficiency except blindness. Degeneration of the germinal epithelium and absence of spermatozoa in the epididymis has been noted in bull calves maintained on a ration low in vitamin A until about one year old (Sutton, Krauss, and Hansard, 1940).

The reproductive system is somewhat more sensitive to lack of vitamin A (and E) than the rest of the body and apparent good health is not a safe guide (Friedman and Turner, 1939).

From feeding experiments in rams, Gunn and co-workers in Australia concluded that lack of green feed, with its associated likely vitamin A deficiency, was the cause of observed degenerative changes in rams' semen at times of the year other than those of intense heat, and that pasture deficiencies were liable to be the cause of various degrees of seminal degeneration wherever green food was scarce or unavailable for long periods, especially in prolonged droughts. In South Africa, Quin (1938) considered that vitamin A deficiency was the only important vitamin deficiency, especially in dry winter pastures. Myburgh (1940) obtained low carotene values for the natural grasses of the veldt in South Africa during the cooler months of the year, when the rainfall was low, which gives a strong indication of a likely vitamin A deficiency during this period. The intensity of green colour in fodders is regarded as a good indication of their carotene content (Wall, 1940).

In the experiments of Gunn and colleagues the control diet per ram per day consisted of: bran (0·3 lb.), oats (0·4 lb.), linseed cake, broken (0·3 lb.), good wheaten chaff (1·1 lb.), and lucerne hay (0·7 lb.); this was adequate for the maintenance of normal spermatogenesis and for inducing recovery from seminal degeneration of dietary origin. Seminal degeneration occurred in previously normal rams fed on mature and leached pasture hay, or on a basal diet of chaffed straw, sugar, linseed cake, and salt. Seminal degeneration was observed in rams whose body weights remained constant, as well as in those losing weight, while normal spermatogenesis was maintained in some rams despite considerable loss of weight. Night blindness always accompanied seminal degeneration and in most cases it was observed shortly before signs of seminal degeneration became evident. Seminal degeneration of dietary origin was prevented, improved, or cured by feeding a mixed vitamin concentrate consisting of a concentrate of vitamin A (containing as little as 435 I.U. of vitamin D), liver meal, and molasses, carrots and green grass. Feeding young pasture hay led only to late development of seminal degeneration or to none at all. They have suggestive evidence that pasture hay becomes less valuable in preventing seminal degeneration the longer it is kept after harvesting, which may be linked with Smith's (1936) findings of only 25% of the original vitamin A value of hay remaining after 12 months' storage. There was no indication of vitamin C deficiency in rams with seminal degeneration.

Seminal degeneration occurred after about 2 to 4½ months on the deficient diets, and recovery after suitable supplements to the diet required about 2 to 3 months. The seminal degeneration resulting from diet deficiency differed markedly from that caused by seasonal heat and fever, where separation of sperm heads from their tails is the predominant early change. Free heads were present and were particularly numerous in the very earliest stages of degeneration in the semen of rams on deficient diets, but abnormal spermatozoa together with various types of germinal epithelial cells were the most characteristic features and were present in very great numbers in comparatively early stages of degeneration.

Vitamin B.—There is no clear evidence that gonadal injury in mammals can be attributed to lack of vitamin B (Mattill, 1927; Evans, 1928). In rats so severely depleted of this vitamin that they exhibit complete paralysis and other manifestations of advanced beri-beri, motile sperm occur in the epididymis and the testes remain normal. In contrast to mammals the testes of birds are susceptible to lack of this vitamin (*see* Mason, 1939).

A marked atrophy of the prostate and seminal vesicles has been observed in vitamin B_1-deficient rats, though the testes were unaffected (Moore and Samuels, 1931). This atrophy is attributed to a state of partial starvation, which inhibits endocrine activity in the pituitary and testis, thus lowering the testis hormone output to a level insufficient to maintain the accessory sex glands.

Vitamin B deficiency causes a decrease in energy intake. Friedman and Turner (1939) stated that it is doubtful whether the vitamin B complex affects reproduction beyond its effect on food intake, so in view of the difficulty of producing vitamin B deficiency in the larger domestic animals and of the abundance of this vitamin in the common stock feeds, there is probably little need to be concerned about its deficiency in the breeding of farm animals.

Vitamin C.—Ascorbic acid is probably concerned in cellular metabolism. An extraneous source of this substance is required only in the guinea-pig, monkey, and man. Other animals probably synthesise it themselves. Mason (1939) stated that it appears that a deficiency of vitamin C in the

guinea-pig, and probably also in the monkey and man, causes no specific impairment of reproductive structure or function in the male, except in so far as physical debility of the severely scorbutic animal may reduce copulative power.

P. H. Phillips and co-workers (1940) have, however, demonstrated the importance of ascorbic acid for reproductive functions in the bull. The semen of good breeding bulls contains from 3·0 to 8·0 mg. ascorbic acid per 100 ml. fresh semen ; bulls with low fertility show less than 2 mg. ascorbic acid per 100 ml. fresh semen and in some cases only a trace. These workers found that the subcutaneous injection of ascorbic acid (1 to 2 gm. twice weekly for 4 to 5 weeks) resulted in restoring the fertilising capacity of certain impotent or infertile bulls; 29 bulls were treated, of which 4 failed to respond. This therapy seems to be favourable in young growing bulls or heavily used mature bulls, which have difficulty in " settling." It has beneficial effects on both sexual interest and quality of the semen. In general the semen was changed from the thin watery type to thick creamy semen with highly motile spermatozoa. In treated bulls the longevity of spermatozoa in yolk buffer was increased. In one bull, ascorbic acid, which was absent from the semen prior to treatment, began to appear after 2 to 3 weeks' treatment and at the end of 5 weeks the ascorbic acid content of the semen reached the normal level. The increase in the ascorbic acid level was accompanied by a change in the semen from a thin watery ejaculate with no motility to a high quality type which proved fertile. One bull, which failed to respond to ascorbic acid treatment, returned to normal semen production when cod-liver oil was also given. The cause of the variation in the ascorbic acid level in the semen of the bull is not clear. It may be affected by diet, for a low blood plasma ascorbic acid has been found in cattle on a restricted dietary regimen (Phillips, P. H., *et al.*, 1938). Also, regular heavy service appears to tax the resources of the bull and exogenous sources of ascorbic acid are needed. Two bulls, for example, improved by ascorbic acid injections, relapsed a few weeks after treatment was suspended. High ascorbic acid levels, 8·0 mg. or more per 100 ml. of fresh semen, were found in bulls with unreliable breeding records. It is of interest to note that ascorbic acid injections in one such bull reduced slightly the ascorbic acid content of the semen and increased the blood ascorbic acid, as well as causing a marked increase in the longevity of the spermatozoa. Phillips and co-workers concluded that ascorbic acid is intimately involved in the production of virile spermatozoa in the bull and in some manner is vitally concerned in the physiology of reproduction in this animal. It is clear that ascorbic acid is a potent weapon for the treatment of infertility in the bull, but it is highly desirable that its use in practice should be checked as far as possible by plasma and semen analysis for ascorbic acid. On the available data the blood plasma values appear to be of rather more use than the semen values. It is the increase in the plasma ascorbic acid content following ascorbic acid therapy rather than the actual level which appears important, and such increase is associated with a marked improvement in the longevity of spermatozoa.

An interesting development of this work is the observation that the feeding of chloretone (chlorbutol, B.P.) to cattle increases the blood plasma ascorbic acid level (Bortree *et al.*, 1941). Chloretone fed at the rate of 5 gm. per day (this has been continued for 4 months without ill-effect) has a beneficial effect on both sexual interest and fertility (Scheidenhelm *et al.*, 1942). With regard to sexual interest, two bulls required 10 to 15 minutes to serve a cow before treatment ; one bull served in 5 minutes after 3 weeks' treatment and in the other there was continuous improvement until at the end of the 4th month it served immediately. The effect of chloretone feeding on fertility was equally marked. Two bulls, which required 8 and 8·5 services per conception in the 2 months before treatment, required only 2·33 and 2·66 services per conception respectively in 2 to 3 months following treatment. A third bull, which required 5·33 services per conception during 7 months before treatment, required only 1·50 services per conception during 6 weeks following chloretone feeding.

A relationship between vitamin A and vitamin C has been noted in dairy calves (Boyer *et al.*, 1942), which indicates that impaired synthesis of ascorbic acid is the cause of the lowered blood and tissue vitamin C content observed in vitamin A deficiency. Erb and Andrews (1942) have suggested that ascorbic acid may be essential for the action of gonadotropic hormone and that simultaneous administration of the vitamin and the hormone might be successful in inducing ovulation or spermatogenesis when the hormone alone had proved ineffective.

Vitamin E.—Vitamin E deficiency causes testicular degeneration in the rat and mouse (*see* Mason, 1939). Evans, Burr, and Althausen (1927) recognised the following stages in the development of sterility : (1) sperm normal in number, motility, and morphology, but fertilising capacity lost, (2) motility lost, but morphology normal, (3) fusion of sperm in groups, (4) absence of sperm, (5) loss of power to form vaginal plug, and (6) loss of all sexual interest.

The testicular changes in vitamin E deficiency in the rat are unique, in (1) the irrevocable nature

of the histological injury from the time of its early inception, (2) the extensive nuclear changes in the germ cells, and (3) the completeness of the removal of the germ cells from the germinal epithelium (Mason, 1939). The continuation of the degenerative process to completion in all or in the majority of tubules indicates, according to Mason, a profound physiological disturbance in the germinal epithelium preceding the morphological changes. The apparent recovery of normal spermatogenesis in male rats that had been treated after a period of vitamin E deficiency was shown by Mason to be due to the recovery of a limited number of tubules that had not undergone complete degeneration.

Vitamin E deficiency apparently has no effect on the accessory organs, any atrophy in which can be attributed, after prolonged maintenance on the experimental diet (Kudrjašov, 1936), to retarded growth and constitutional inferiority of the deficient rats.

In the light of present knowledge it is doubtful if vitamin E deficiency is of importance in the larger animals. Friedman and Turner (1939) stated that although definite cases of a natural deficiency of other vitamins are recorded for man and animals, there is on record no known instance of a vitamin E deficiency occurring in nature, and this is undoubtedly a reflection of the widespread distribution of this vitamin in all natural foodstuffs.

Season

Seasonal changes in the semen have been observed in Shropshire and Hampshire rams in America (McKenzie and Berliner, 1937) and in Merinos in Australia (Gunn *et al.*, 1942). In Kenya seasonal variations in the semen of both bulls and rams have been noted (Anderson, 1941*a* and *c*, and unpublished data). Seasonal changes have been noted in the semen of the bull in America (Erb, Andrews, and Hilton, 1942). These changes appear to be associated with, or are actually caused by, atmospheric temperature and/or nutritional deficiencies, though other as yet unexplored factors may also be involved.

The differences between breeds and between individuals in the reaction of the testis and sperm to temperature may be considered as a problem of adaptation to environment (McKenzie and Berliner). Kronacher (1937) attributed this adaptability to genetic make-up. Nichols (1933) has studied the problem of adaptability in sheep. McKenzie and Berliner's observations on Hampshire and Shropshire rams suggest that Hampshires may be, from the standpoint of breeding efficiency and capacity, better suited to conditions at Columbia, Missouri, U.S.A., than Shropshires, and that differences in degrees of resistance to heat may be breed characters. The Hampshires may be considered as adjusted to the very high temperatures of summer, and the Shropshires as never having had the same capacity or as having lost it.

According to Duerst (1931) different breeds of cattle are the product of constitutional factors, based on the activity of endocrine glands. Spöttel (1929) noted conspicuous breed differences in thyroid activity of sheep in Europe and changes in histological appearance in different seasons. The possibility, suggested by McKenzie and Berliner, that Shropshire rams belong to what Duerst called a hypothyroid strain, and that Hampshire rams belong to the balanced thyroid type, appears to have been confirmed by Berliner and Warbritton (1937).

Ram

There is now a considerable amount of evidence to indicate the natural conditions under which heat degeneration of the testis in the ram is likely to occur. Phillips and McKenzie (1934) observed that the increase in the dependence of the scrotum by relaxation of the tunica dartos muscle, which is the main method of controlling local heat loss, reaches its maximum at a temperature of 72·5° F., which suggests that the temperature at which rams become susceptible to the effects of heat is low. Gunn and co-workers (1942), from their experimental data and observations on seasonal changes in the semen of the ram, anticipate that the adverse effects of heat may be noticeable in districts in which daily recurrent maximum temperatures are for long spells above 90° F., with occasional readings of 100° F., and that such effects will be well marked in districts subject to daily recurrent temperatures for long spells above 100° F. Their data also show that the lower the daily minimum temperatures, the less is the harmful effect of high daily maximum temperatures on spermatogenesis. This latter observation is important for tropical plateau countries, such as Kenya, in which there is a large diurnal range in temperature.

America

For their estimation of libido in Shropshire and Hampshire rams, McKenzie and Berliner (1937) took the number of attempts to copulate and the number of completed copulations in a given

period. There was no season during the year when the rams showed no mating desire, unlike the ewes during the anoestrous period. Their observations, in fact, suggest that breeding desire does not change markedly throughout the year, but that during February, March, and April the rams apparently do not complete copulation as readily as at other times. During these months erection of the penis seemed lacking or imperfect, resulting in failure of intromission ; the rams continued to mount the ewes and attempted to serve them so that with failure of gratification the number of attempts to copulate increased. This should not be interpreted as increased mating desire. The number of completed copulations suggests that a period of increased mating ability and mating desire occurs from September to December.

A marked decrease in sperm production was observed over a longer period in the Shropshires than in the Hampshires. August and September were the low months for the Shropshires, and July for the Hampshires. In the Shropshires, the period of high sperm production lasted from October to January, and in the Hampshires from August to January. A comparatively high level of spermatogenesis was found in both breeds in the spring, but it was higher in the Hampshires than in the Shropshires. There was a marked variation in sperm production in the two breeds, and in individual rams within the breeds. Hampshire rams produced a more concentrated semen throughout the year than did the Shropshires.

There was no difference in the ejaculate volumes between the two breeds. The largest total volumes of semen in both breeds were collected in September to October. It seems that the number of spermatozoa was dependent not so much on the volume as on the concentration of spermatozoa and the number of services during each test period.

Green (1940*b*) observed that density (billion/ml.) of Shropshire ram sperm, which was 3·5 in May, declined from June to August, when it reached 1·36, rising thereafter to 3·41 in December. He concluded that more attention should be given to the question of the ability of rams to recover before the breeding season from lowered seminal quality due to summer environment.

In the Shropshires the increase in the number of abnormal forms per 1000 spermatozoa was very marked (maximum 733 per 1000) in June, July and August, but in the Hampshires was noticeable (220 per 1000) only in July. The absolute number of abnormals increased from February to August in the Shropshires. The total number of spermatozoa was still small in August to September, but there were three times as many normal spermatozoa as at the preceding test period. The abnormal sperm dropped to a very small fraction of the whole during the following breeding season. The proportion of abnormal sperm was at all times lower in the Hampshires than in the Shropshires. Comparison of the normal sperm production with the total sperm production shows that the Shropshires have a short season in the year, during August to September, when they are temporarily sterile, followed by a fairly rapid recovery and high spermatogenetic activity during the breeding season. Such a distinct non-breeding season was not shown by the Hampshires (McKenzie and Berliner, 1937).

Green (1940*b*) studied the seasonal trends of sperm cell types in the semen of 12 purebred Shropshire rams, starting when they were 10 months old and continuing for 1 year. A steady decline in sperm quality was apparent from January, when normal cells numbered 230/500, to mid-July when they were 160/500, after which they rose again to 400/500 in November. During June and July the ejaculates presented an increased number of head abnormalities (38/500), tailless heads (189/500), and minute sperm (46/500), as compared with 20/500 head abnormalities, 118/500 tailless heads, and a very few minute sperm in the earlier months of the year. Considerable improvement in quality occurred between July and October, head abnormalities declining to 10/500, tailless heads to 40-60/500, and minute sperm to 2/500. All the sperm types remained relatively stationary thereafter.

Summarizing these observations of McKenzie and Berliner, a distinct period of increased spermatogenetic activity was observed in both breeds (for the Shropshires in October to January and for the Hampshires in August to January), characterised by (1) a large number of completed copulations, (2) a large number of spermatozoa ejaculated, (3) large total volume of the ejaculates and (4) the occurrence of small absolute and relative numbers of abnormal spermatozoa. In the late winter and throughout the spring months, the number of spermatozoa ejaculated, the volume of the ejaculates, and the concentration of spermatozoa were smaller than in the breeding season. In the Shropshires the number of abnormalities was higher than during the breeding season, but probably not high enough to impair fertility. There were fewer services completed during this period than in the breeding season. There was a period of decreased sperm production, extending from June to September, very pronounced in the Shropshires, but only slightly noticeable in the Hampshires and then principally in July. The performance of the Shropshires during this period was

characterised by (1) a small number of spermatozoa ejaculated, (2) a decrease in the concentration of spermatozoa, and (3) a marked increase in the absolute and relative numbers of abnormals.

Brady and Gildow (1939) found that in sperm collected in the spring from Suffolk rams, compared with winter collections, the motility was 23% less, concentration 38% lower, motile cells 17% less, activity 25% lower, and viability and *p*H practically identical.

Lambert and McKenzie (1940) stress the importance of avoiding excessively high temperatures throughout the summer. They cite unpublished data from the Missouri Agricultural Experiment Station, which indicate that Shropshire and Hampshire rams, protected from temperatures above 80° F., produce more semen and a larger number of normal sperm, and are prepared to enter the breeding season earlier than rams subjected to the usual temperatures of 80° to 100° F.

Australia

Gunn and co-workers (1942) observed seasonal changes in rams' semen which varied according to atmospheric temperatures. Hot weather caused seminal degeneration and the cooler season favoured recovery. There were minimal changes in the absence of extremes of heat. The seasonal changes varied from slight in the early stages and in the cool districts (best indicated by activity numbers, longevity, and sperm numbers) to well-marked degeneration including considerable morphological changes in the later stages in the hotter districts. The degree of seasonal seminal degeneration varied in different individuals, but in hot districts it might be so marked that normal spermatozoa were entirely absent or were present only in very small numbers. It is probable that such individuals were temporarily impotent. Seasonal degeneration was followed by a peculiar recovery stage and finally by optimum semen production. Although a number of causes might produce seminal degeneration, including deficient nutrition, it was considered that the main cause of the seasonal changes in rams' semen was high atmospheric temperature. It appeared probable, from a consideration of the morphological changes in rams' semen alone, that the percentage fertility of rams might vary with the seasonal heat in the hotter districts. The degree of the changes and the dates of their inception and of recovery from them varied with the districts. British breeds of sheep reacted to heat similarly to Merinos.

These workers stress the value of shade in preventing seminal degeneration due to atmospheric heat, and this view is supported by the opinion of graziers in Australia, who state that the fertility of sheep kept in shadeless hot districts and paddocks is considerably lower than in those kept in well-shaded, even though hotter, areas. Further, experienced sheep men in Western Australia have observed that even the habits of sheep aim at loss of heat, since in summer on windless days they invariably spend the heat of the day in the shade, whereas when there is a wind they go on the high ground irrespective of shade.

Gunn *et al.* state that under the rather special conditions of animal husbandry which exist in Australia on most properties on which Merinos are bred, diet deficiencies of a seasonal nature, now shown by them to be of utmost importance in spermatogenesis, are likely to be of rather common occurrence, despite the sheep maintaining their condition ; in some districts such deficiencies may be annual, seasonal occurrences, and in others they may occur only, or mainly, during drought.

The interrelationship of dietary conditions and atmospheric temperatures with seasonal changes in rams' semen is illustrated by the effects in hot areas with monsoon rains and in those relatively independent of them. Rams kept in the former conditions (Central Queensland), where the greenest feed is present early in the year and then rapidly deteriorates, are prevented from recovering by seasonal heat until early in the autumn, and can maintain normal spermatogenesis for a few months only on account of the rapidly diminishing value of the available pastures from June on ; whereas rams kept in hot areas relatively independent of monsoon rains, where green food is most plentiful in the cooler period of the year, return to normal semen production in the autumn after the heat of summer, and remain normal until the heat of the next summer again causes seminal degeneration, often while plenty of green feed is still available.

Gunn and co-workers on the basis of their findings on marked seminal degeneration in the semen of rams kept in artificial hot atmospheres, and on the occurrence of seminal degeneration in summer in rams in the hotter districts, refer to the importance of selecting the correct season of the year for mating (*see* p. 17).

Russia

Buchman and Andreevskiĭ (1940) studied the variation of sperm production and thermo-regulation of rams in summer. Observations were made on 3 rams grazed on the open steppe in Askania Nova during the whole day and on 4 grazed only in the early morning and the late evening. The average

temperature in the open was 30·2° C. and this had a markedly harmful effect on sperm quality, as shown by reduction in volume of ejaculate, lower density, and greater proportion of abnormal types. The deterioration of sperm quality did not go beyond a certain limit, which varied in individual rams, after which improvement set in. Deterioration of sperm quality was also, however, observed in rams kept in the shed where the temperature range was 27°-32° C. Rams kept on pasture showed during the day a certain disturbance of the thermo-regulatory mechanisms of the body and a more marked one of the scrotum.

Bull

In Kenya a seasonal variation in the motility and volume of the ejaculates of the bull has been noted (Anderson, 1941*a*). Both, but particularly the volume, showed a decrease approximately from May to August. A slight monthly variation in the *p*H of semen, which was not, however, statistically significant, was observed for the 6-month period from August to January inclusive ; the *p*H fell from 6·84 in August to 6·61 in November and rose slightly in December and January (Anderson, 1942*a*). It is possible that a greater seasonal variation would be observed over a longer period which included the months when a depression in volume and motility has been observed.

Erb, Andrews, and Hilton (1942) have recently studied the seasonal variation in the semen quality of the dairy bull, using regular herd sires in Purdue University Dairy Herd, Lafayette, Ind., U.S.A. Highly significant differences between months were noted for all factors studied—initial motility, volume, concentration of sperm per mm.^{3}, total sperm, abnormal sperm per 1000, survival period and total motility rating—except the *p*H, and this showed variation only at the 5% level. The average semen volume and the average initial motility were least in July, August, and September. The average concentration of sperm and total sperm per ejaculate were greatest in April, May, and June. The average period of sperm survival was least in August, and lower in July, September, and November than during other months. The average number of abnormal sperm was 25% greater during July, August, and September than during the next highest month of the year. In general, the quality of semen produced by the bulls in this study was significantly superior during the spring and significantly inferior during the summer. Semen produced during the autumn and winter months did not vary significantly from the mean. Since the management and plane of nutrition of the bulls were similar throughout the experimental period, it was concluded that the changes observed were the result of those factors which characterise the seasons—temperature, light, relative humidity, and other general or obscure atmospheric factors. (*See also* p. 137.)

Other Conditions

Dipping and Shearing.—Gunn and co-workers (1942) demonstrated that absorption of arsenic occurs through the skin when rams are dipped for a minute or less in a dipping fluid containing less than 0·2% As_2O_3 and that arsenic absorbed into the system of rams is capable of causing marked degeneration of the sperm. The longer the fleece and the hotter and more humid the weather at and after the time of dipping, the more severe is the seminal degeneration. Seminal degeneration caused by arsenic takes longer to develop, and recovery from it is slower, than that which has been brought about by most other causes. Since these workers found that dense fine-woolled rams are more susceptible to seminal degeneration resulting from dipping than are Border Leicesters and primitive hairy types of sheep, it is suggested that the extreme denseness and fineness of the wool of Merinos increases the amount of fluid held in the fleece, as well as the time taken for it to dry out, and that the " condition " (grease) of the fleece, acted upon by the alkaline dipping fluid and becoming saponified, or in some way helping to form an emulsion with the dipping fluid, induces more perfect contact of the dip with the skin.

Gunn and his colleagues recommend, to avoid the occurrence of seminal degeneration with consequent probable reduced fertility (1) that rams carrying long fleeces should not be dipped in strong arsenical dips for 2 months prior to mating, or if such dipping is necessary, they should be previously shorn, or mating should be delayed until about 8 weeks after dipping, and (2) that it is unwise to dip rams in arsenical dips immediately after shearing, since arsenic may be absorbed through the cuts made at shearing, thus dipping should be postponed until about a week or so later, when most wounds will have healed.

Dipping in rather cold, showery weather in a saponified phenol type of fluid dip, guaranteed to contain 1% tar acids and diluted 1 to 110 in cold water, has no effect on ram's semen whatever. There is no evidence of any deleterious effect of shearing on spermatogenesis (Gunn *et al.*).

Footrot.—Gunn and co-workers have observed seminal degeneration in certain clinical conditions,

unassociated with high fever, such as contagious footrot. Webster (1937) has reported reduced fertility in rams affected with footrot.

Abscesses.—Subcutaneous injection into rams of pus from acute abscesses caused rapid seminal degeneration similar to that seen in fly strike (Gunn *et al.*). The degeneration depended on the degree of fever caused and possibly also on the abscess formation. The separation of the heads of spermatozoa from the tails was the most prominent feature. After opening of the abscesses the semen quickly returned to normal unless the abscess persisted for a long time or sinuses resulted. The intraperitoneal injection of pus caused acute fever, and consequent seminal degeneration was marked and persistent. The separation of heads from tails was followed by the appearance of an increasing proportion of whole but abnormal spermatozoa together with types of germinal epithelial cells.

These workers have noted the infrequent occurrence of abscesses in the cavity of the tunica vaginalis. They believe that the frequently occurring adhesions between the parietal and visceral vaginal layers in many cases have resulted from inflammation from a previous infection within the vaginal cavity. All stages from acute suppuration associated with mixed bacterial infection (including *B. coli* and *B. pyogenes*) to exscapulated collections of pus, and to old firm fibrous adhesions have been found. Rams thus affected commonly show seminal degeneration similar to that caused by intraperitoneal injections of pus.

Infestation of rams with oesophagostomiasis produced no change in the semen, but such rams were more susceptible than normal rams to other causes of seminal degeneration, such as nutritional deficiencies. Severe infestation with *Haemonchus* or *Trichostrongylus* did not interfere with spermatogenesis.

Trauma.—Injury to the scrotum causing inflammation, particularly if associated with oedema, is a fruitful cause of seminal degeneration. Extensive infected wounds elsewhere have also been found to cause seminal degeneration.

Toxins, etc.—The injection of bacterial toxin (*C. welchii* type), a vaccine (from *C. pyogenes*), histamine, and foreign protein (sterile milk) and horse serum, produced at the most only a slight rise in body temperature and only minor changes in the semen typical of early febrile conditions.

Clinical Abnormalities of the Genitalia

Balanitis, either the typical infectious condition known as " pizzle rot " or simply inflammation and general infection due to irritation set up by foreign bodies such as grass seeds, has not been found to be associated with any special seminal abnormality (Gunn *et al.*).

Eczema of the scrotum, scalding and local inflammation and infections resulting from wounds, injuries and fly strike are not infrequently seen and are often associated with some degree of seminal degeneration.

Epididymitis.—This is one of the most common genital abnormalities in the ram (Gunn *et al.*). The tail is most frequently affected but the head and the whole body may be involved. Indications have been found that it begins in a tiny spermatocoele (cyst containing spermatozoa), apparently by the breaking down and coalescence of epididymal tubules, the area being surrounded by a zone of fibrous tissue. In very long-standing cases much atrophy of the testicular tissue is present. Sometimes the pathological process spreads quickly to the testicular tissue, causing fibrosis and even calcification. The disease occurs equally in mated rams and in those which have never served. There is no proof as to the actual cause.

Even before the disease can be determined by palpation, changes occur in the semen, less pronounced but otherwise characteristic of the semen of rams clinically affected with epididymitis, namely, separation of sperm heads from their tails and the occurrence of large numbers of multi-nucleated young germinal epithelial cells. The seminal changes become progressively more marked after the disease becomes clinically apparent. In most long-standing cases, particularly if unilaterally affected, the semen contains but few normal spermatozoa, together with a variable number of germinal epithelial cells and degenerated spermatozoa.

In one ram marked semen changes were observed for a period of 2 years, after which the semen gradually returned to normal and the ram " recovered " as far as its semen was concerned, though it still remained an obvious clinical case of epididymitis. This ram had a unilateral affection only, and it is suggested that the eventual return of the semen to normal was associated with complete obstruction of the epididymis on the affected side, thus permitting the passage of spermatozoa only from the other normal side. In only a small minority of many typical cases of well-developed epididymitis has the semen approximated normal and these are regarded as " recovered " cases. It is considered

that only a small proportion of cases ever recover. All cases of epididymitis whose fertility was tested were sterile, but it is anticipated that the small proportion of affected rams which " recover " after a number of years would be fertile. But it is doubtful if it is ever profitable to retain such rams for breeding except possibly in the case of unilaterally diseased rams in which the affected testis is removed surgically.

Hypoplasia and Atrophy of the Testis.—In cases in which there is a temporary decrease in size and an increase in flabbiness of the testis, due probably to decreased spermatogenesis as suggested by Gunn and co-workers, recovery may occur on removal of the cause. These workers have noted that a condition of hypoplasia or atrophy, usually unilateral and of a permanent nature, is relatively common in rams. In such cases the cord is usually short and well drawn up. The consistency is either soft and flabby (on section fine-grained and muscle coloured) or hard and indurated (sclerosed and often calcified). Often the epididymis is ill-defined. They have observed that the semen of such rams remains abnormal for very long periods, and have never known it to return entirely to normal. It usually contains a proportion of sperm heads separated from their tails, often a percentage of other abnormal spermatozoa, and always a number of small, round, often nucleated, germinal epithelial cells. They state that the possible affinity of testis hypoplasia with unilateral cryptorchidism is suggested by the well-known fact that in some rams one testis descends much later than the other. Cryptorchidism in the ram is hereditary (Warwick, 1931) and hypoplasia in the bull, apparently a similar condition to that seen in the ram, is also hereditary (Lagerlöf, 1938).

General.—The following incidence of clinical abnormalities is based on the examination of over 9000 rams in Queensland and New South Wales (Gunn *et al.*): epididymitis and spermatocoele 5·4%, hypoplasia or atrophy of the testis 3·4%, varicocoele 1·4%, cryptorchidism (unilateral) 0·4%, inguinal hernia 0·2%. Thus, over 10% of rams examined were found to be clinically abnormal.

The Bacteriology of Semen

Bacteriological studies have been made on semen recovered from vaginas of cows immediately after service, after douching and disinfecting vaginas and sheaths (Gilman, 1922 ; Webster, 1932). As Gilman says, this method is not entirely satisfactory because of possible contamination from the vagina. He found micrococci, haemolytic and non-haemolytic streptococci, coliform organisms and *B. abortus*, and considered that the genital tract and semen of normal bulls contained few if any bacteria, but that sterile bulls or those of diminished fertility usually had large numbers. Webster (1932) found that on culture normal semen yielded diptheroids and micrococci, while bulls from herds in which enzootic sterility was present produced semen which contained in addition many haemolytic streptococci. Gilman (1921-22) isolated *Pseudomonas pyocyaneus* and unidentified rods, as well as micrococci, streptococci, and coliform organisms ; the results indicated that the genital tracts of normal healthy bulls were either free from bacteria or contained very low numbers, whereas impotent bulls harboured large numbers of organisms which were undoubtedly ejaculated with the semen. Hatziolos (1937), who used the artificial vagina and tried to collect semen as free as possible from contamination, found organisms consisting mainly of coliforms, the proteus and pseudomonas groups, cocci, and spore forming rods in every ejaculate.

In a recent study, Gunsalus, Salisbury, and Willett (1941) found in 43 ejaculates collected from 19 bulls with the artificial vagina, a bacterial count of from 1 thousand to 22 million per ml. All the types of organisms reported by other workers, with the exception of streptococci, were found. Diptheroids occurred most regularly in large numbers ; others found included *Pseudomonas*, *E. coli*, staphylococci and bacilli. Contamination from bedding, manure, soil, and unsterilised glassware would include *E. coli*, bacilli, and staphylococci. These types could be eliminated or markedly reduced in number by attention to cleanliness, such as douching the sheath with sterile distilled water and observing precautions in the preparation of diluting fluids, when sperm is stored. Diptheroids, on the other hand, were always present in the semen in variable numbers, no matter what precautions were taken. *Pseudomonas pyocyaneus* was isolated from 5 of the 19 bulls. In these 5 bulls were included 3 of the 4 bulls dropped from breeding operations of the Central New York Artificial Breeding Co-operative ; of the other 2 bulls, one became sterile and the other had a record of 12·1% conceptions for 91 inseminations by artificial breeding.

The presence of bacteria in semen is also of importance when semen is stored. Roemmele (1927) found bacteria in semen stored at room temperature and at 9° C. The rapid growth of bacteria in semen, especially a small motile bacillus which formed a blue-green pigment (probably *Pseudomonas pyocyaneus* according to Gunsalus *et al.*) was noted (Krzyszkowsky and Pawlow, 1927) but there was

no indication whether the bacteria had a detrimental effect on spermatozoa. Gunn (1936) detected no influence of bacteria on the survival of spermatozoa stored at 4° C. Bacterial growth was observed by Hammond (1930) in samples stored at 10° C., but there was no greater incidence of infection in female rabbits inseminated with semen in which large numbers of bacteria had developed than with samples where fewer organisms had grown due to a lower storage temperature. Gunsalus, Salisbury, and Willett describe a method for the preparation of almost sterile yolk-phosphate diluent. Unless precautions are taken the diluent may be responsible for the addition of large numbers of bacteria to semen samples. During storage bacterial growth is held at a minimum by storing at 5° C. or lower.

Frequency of Copulation and Ejaculation

Present information on the frequency of copulation and the reproductive capacity of males serves to indicate the degree to which males may be used in intensive breeding programmes. While considerable differences exist between species and between individuals of a species, it seems that without affecting breeding performance to any extent, much greater use than was hitherto supposed can be made of males of those species, such as bull and ram, which produce relatively small ejaculates. Less intensive use is possible with species such as the horse, boar, and dog which produce a large volume of ejaculate at each mating. Further, it would seem that in some animals at least, mating and ejaculation stimulate sperm production. Walton and Edwards (1938) consider that the "exhaustion test"—the successive collection of a number of semen samples—forms the best means of measuring male fertility.

Bull

The effect of frequency of collection can be examined in two groups, (*a*) in which there is an interval of minutes between collections of semen, and (*b*) in which there is an interval of days. Kirillov and Morozov (1937) compared sperm production at the 1st and subsequent matings on the same day and found that it was inferior at the 1st mating in respect of all characters, except volume of ejaculate and number of spermatozoa. The variability of all characters was greatest at the first mating. Davis and Williams (1939) compared the 1st, 2nd and 3rd ejaculates of 11 bulls for volume of semen, percentage of progressive motility of spermatozoa, concentration of spermatozoa, and *p*H. The volume remained nearly constant for all ejaculates with notable differences between individual bulls. The ejaculates ranked as follows: (1) for motility—second, first, third; (2) for concentration—first, second, third; and (3) for *p*H—first, third, second. The second ejaculate had a slightly larger volume, a slightly higher percentage of progressive motility, a lower concentration of spermatozoa and a slightly higher *p*H, and proved slightly more effective for insemination. Herman and Swanson (1941) examined two ejaculates collected at 5 minutes' interval. The second ejaculate had a larger volume, a higher concentration of spermatozoa, a better motility, a lower *p*H, and a greater number of abnormal spermatozoa. They found that the difference in *p*H was subject to extremely wide individual variation and in many instances the *p*H of the second ejaculate was higher than that of the first. In 2 cases the motility of the 1st ejaculate was better than that of the second, but often there was no difference. Most of the average increase in abnormal spermatozoa in the 2nd ejaculate was due to an increase in coiled tail forms, caused by one bull. In general, some bulls showed little difference between the two ejaculates. In others the first was thinner and more watery with a lower concentration and motility of spermatozoa. They suggest that quickness of service may have accounted for the poorer quality of the first ejaculate. It appears that a *p*H of 6·80 or higher in the 1st ejaculate may be associated with an abnormally low concentration of spermatozoa. Anderson (1941*b*, 1942*a*, 1942*c*) compared two successive ejaculates collected within a few minutes of each other from fertile and sterile bulls. In fertile bulls the volume and motility improved in the 2nd ejaculate. Even in bulls in which the semen was considered of poor quality there was some improvement in the 2nd ejaculate, though in some cases volume and motility were less. The second ejaculate had a greater concentration of spermatozoa and a lower *p*H. In bulls with epididymitis, the volume, which was much the same in the first 2 ejaculates, increased slightly in the third. In sterile bulls with epididymitis or small testes the *p*H became increasingly alkaline with successive ejaculates. In such cases it is probable that the functions of the epididymis and testis are depressed and successive ejaculates from such bulls probably contain a relatively and increasingly smaller epididymal fraction and for that reason are more alkaline. An increase in the *p*H of successive ejaculates has also been noted in some bulls which clinically appear to have normal testes and epididymides. Bearing in mind the inverse relationship noted between *p*H and other semen properties, an increase in the *p*H in the 2nd ejaculate probably indicates the poorer quality of that semen.

It seems that a considerable number of matings can be made by the bull in a relatively short time without greatly affecting the quality of the semen. Kirillov (1933-34), and Kirillov and Morozov, (1933, 1937) concluded that (1) it is practically impossible to exhaust the bull, (2) sperm is produced continuously and regeneration requires less than 24 hours, and (3) mating stimulates sperm production. Two matings a day appear to be the optimum number. The sexual impulse increases with the number of matings. An example of the maximum possible degree of sexual activity was that of 24 matings in 27 hours. The concentration remained at the same level up to the 15th mating, after which there was a sharp fall. After a rest of 18 hours the level was again raised and persisted till the 23rd mating, when it began to fall. Similar results were obtained with bulls which performed 11 matings a day. Lagerlöf (1934) allowed a bull to serve 29 times in 19 days, and although there was a decrease in the number of sperm in some ejaculates, a night's rest was sufficient to restore the normal number the following day. Kirillov and Morozov found that with bulls performing many matings a day, a day of rest had a beneficial influence on sperm production, but only during the few following days. Lagerlöf observed that bulls differed in the number of matings they could give per day without showing a decrease in the concentration of spermatozoa ; sex strain which one bull can stand without harmful result may harm another bull. Verevkina and Smirnov-Ugrjumov (1938) observed a deterioration in semen quality after a large number of daily matings. At the 8th and 18th matings respectively the volume of the ejaculate was 1 ml. compared with 0·25 ml., the number of sperm was 0·7 *v.* 0·075 ($\times 10^6$) and the resistance 680 *v.* 300.

Webster (1932) has noted that the first semen collected after a long period of sexual rest is of poorer quality as regards motility and morphology than a second ejaculate collected within a short time. Herman and Swanson (1941) collected semen from 4 bulls regularly after a prolonged sexual rest (2 and 3½ months). The most outstanding change was the decline in the percentage of abnormal spermatozoa with succeeding ejaculates. The initial motility tended to increase, but the concentration of spermatozoa did not vary uniformly from the 1st to the 9th ejaculate. Anderson (1941*a*) found that the number of days between ejaculations had little or no effect on the motility and volume of the ejaculate. There was a slight decrease in motility and increase in volume as the interval increased from 1 to 20 days, but this tendency was not maintained for longer intervals. In one bull, for example, the first collection after an interval of 7 months was 80% motile. This has also been noted by Herman and Swanson after a 2-months' interval. However, it has been noted on a Kenya farm, using artificial insemination on a big scale, that long intervals between collections of semen tend to lower the motility.

Trimberger and Davis (1942) comparing the first and second ejaculate found a slight advantage in favour of the second, but no significant differences when the previous service had been within 20 days. A marked and significant difference was found if the previous service was outside the 20 days limit. This difference was more pronounced when the previous sample had been taken between 20 to 30 days than when the time interval was over 30 days. After a long rest period more than 2 samples are required to show a return to normal.

Burch (1939) stated that the average bull cannot stand service oftener than once every 3 days over an extended period and still deliver a satisfactory ejaculate. Trimberger (1942) observed that the frequency of use of bulls is an important factor in successful operation of breeding associations and may have an influence on breeding records as well as on successful storage of semen. Two ejaculations every 3rd or 4th day with an occasional rest from sexual activity produce the best results.

Weatherby, Reece, and Bartlett (1940) investigated the question of the ability of dairy bulls to withstand regular service for artificial insemination during the period of a year. 362 semen samples obtained from 5 bulls were tested for volume, concentration, and longevity. In a bull ejaculating every 3rd day for 11½ months the volumes of ejaculates decreased, while concentration and longevity (average 14 days) remained fairly constant. A bull, which ejaculated once a day for 57 days, showed no marked variation in weekly volume of semen but concentration varied from 2179 million sperm per ml. in the 4th week, to none in 5 ejaculates obtained in the 9th week (average for the 57-day period, 1114 million sperm per ml.). Sperm longevity increased up to the 5th week, decreasing thereafter to a very low level. After a 24-day rest the same animal was ejaculated once weekly for the next 11 months, during which period concentration averaged 308 million per ml. higher, and longevity 8 days longer, than in the 24-hourly ejaculates. When collections were made twice weekly, one immediately after the other in 2 bulls, the average volume and motility of the second collections were greater in both animals, while concentration was less for the first collection in one and greater in the other. (*See also* p. 137.)

Ram

The following observations have been made on semen produced under varying degrees of use by rams of a native Caucasian coarse-woolled breed, and by Merino rams (Ljutikova and Polovcova, 1933). (1) With one service daily the average volume of ejaculates was 0·76 ml. containing 1540 million spermatozoa; (2) with 2 services daily at 1½-hour intervals, over a period of 8 days, the first ejaculate contained 1780 millions and the second 1310 millions; (3) with 3 services daily for the same period, the first ejaculate consisted of 1·07 ml. containing 1250 millions, and the third 760 millions, in 0·7 ml. The different ejaculates were used to inseminate 240 ewes. The lambing was 55% in (1), 43% for the second ejaculate of (2), and 47% for the 3rd ejaculate of (3); it was concluded that 3 matings on one day do not cause any exhaustion of the ram, or production of inferior sperm, but that the fertilising capacity is greatest in the sperm produced after a night's rest. Kuznecova *et al.* (1932) found that it was very difficult to exhaust completely the sex organs of the ram, for one ram after 42 consecutive ejaculations within 9 hours still ejaculated 100 million spermatozoa; the first ejaculate contained 2500 million spermatozoa. According to Lambert and McKenzie (1940) it appears that more than 3 matings a day can be made without serious depletion of spermatozoa. Loginova (1936) concluded that with appropriate feeding and management 5 to 7 matings per day are entirely practicable.

McKenzie and Berliner (1937) studied the frequency of copulation (natural coitus) in Hampshire and Shropshire rams. There was a tremendous variation in breeding activity between individual rams within the breeds. Depletion of sperm set in more often in rams that behaved actively, perhaps because they bred more often than did sluggish breeders which stopped copulating before their sperm supply was exhausted. Reduction in the number of spermatozoa was greater on the whole in Shropshires than in Hampshires, and in the non-breeding season than in the breeding season in both breeds. After a marked drop in sperm numbers, recovery was often seen after an hour. Terrill (1937) noted that when one ejaculate was produced in a period of 30 minutes, its value was below the first ejaculate of rams producing 2 or more ejaculates in 30 minutes. Volume, concentration, and degree of motility decreased after the second ejaculate within 30 minutes.

McKenzie and Berliner also studied the effect of excessive copulation by allowing 6 rams eleven 10-minute periods for service in 9 hours. Three Hampshire rams performed 24, 20, and 13 services respectively in this period, and three Shropshires 23, 16, and 13 services. In all cases the number of sperm decreased markedly after the first service. The Hampshires showed no indication of exhaustion, but intermittently the Shropshires copulated without producing semen. One Shropshire showed signs of exhaustion after a few ejaculates of poor quality.

Polovceva (1938) stated that the average number of spermatozoa in the epididymis of the ram was about 162×10^9, of which 76% were contained in the cauda, the distal part of the cauda containing some 58×10^9. Twelve rams were exhausted by repeated service with the artificial vagina. The total count of spermatozoa ejaculated was $49{\cdot}17\pm5{\cdot}71\times10^9$, *i.e.* exhaustion was reached when 84% of the spermatozoa of the distal part of the cauda epididymis had been used.

The data of McKenzie and Berliner confirm the reports of other workers that in normal rams complete exhaustion of sperm is difficult to obtain and that a short rest period is sufficient to bring fresh spermatozoa, if only a temporary lack of spermatozoa is induced. There are individual and breed variations in the rate at which spermatozoa make their reappearance after temporary reduction. Under similar conditions some rams reach a state of exhaustion earlier than others. The time of onset and the duration of exhaustion appear to be roughly proportional to the actual breeding capacity of the ram.

It would appear that there is a certain wave-like progress of spermatozoa from the testis and that differences occur in the rate at which they are pushed forward. The rate in some rams must be so fast that each ejaculate contains a normal amount of spermatozoa, but in others copulation may not result in emission of semen because refilling has not taken place. If a sluggish, slow-breeding ram yields an ejaculate of subnormal quality or none at all after a sufficiently long rest period, this can probably be considered a sign that the refilling did not occur because of lack of spermatozoa in the testis or of some grave disturbance in the neuro-muscular mechanism of sperm transportation.

Frequency of Artificial Ejaculation (Gunn, 1936).—In rams submitted to artificial ejaculation as frequently as 8 times in 10 days, 18 times in 26 days, and 17 times in 45 days, no decrease occurred in the volume of ejaculates, in the percentage of motile spermatozoa, or in the degree or duration of motility. No alteration in morphology or staining reaction was observed. In older rams after frequent stimulation the vasa deferentia and the epididymides were often found free of spermatozoa, whereas

the tubules of the testes, on histological section, were found crowded with spermatozoa. In younger animals less perfect depletion of the contents of the epididymides was demonstrated. This was assumed to be due to a more rapid refilling process from the enormous stores of spermatozoa in their testes. Gunn's conclusions that the refilling process was probably dependent partly on the time available and partly on the animal agree with McKenzie and Berliner's observations.

Gunn suggests (1) that the rapid removal of spermatozoa is a stimulus to their further production, and the absence of periodic emission reduces the rate of production, (2) that spermatozoa are continually being produced in the testes, and (3) that in normal rams submitted frequently to artificial ejaculation repletion of the stores of semen occurs within 24 hours. Frequent ejaculation causes the more rapid passage of sperm through the epididymis. From the greater ease with which ejaculates have been obtained from rams on later occasions of successive artificial ejaculations an increase in the impulse to ejaculate is suggested.

It was noted on many occasions that if even a virile young adult ram be submitted to a series of stimulations and then be allowed a rest interval of only half to one hour before being subjected to a second series of stimulations, though the volume of the second ejaculate may remain normal, the number of spermatozoa contained in it is invariably less than in the first ejaculate, or in an ejaculate produced after a rest period of 24 hours or more. Slow refilling is more likely to occur in an aged or an immature animal than in a young vigorous adult.

After a period of sexual rest an increase in the number of abnormals in the first ejaculate has been noted (Phillips and McKenzie, 1934 ; McKenzie and Berliner, 1937) when the semen has been ejaculated naturally. This increase in abnormals has not, however, been obtained when ejaculation is induced with the electrical method (Gunn, 1936, Gunn *et al.*, 1942), possibly due to the greater number of spermatozoa ejaculated with the latter method.

Stallion

Lewis in 1911 observed that stallions differed greatly, some sluggish or older stallions showing signs of sperm exhaustion after fewer services than younger and more energetic animals. He found a marked decrease in the number of spermatozoa and lowered vitality and viability in the later ejaculates as compared with the first. Similar results were obtained by Polovcova (1927), who found that a rest period of 48 hours was sufficient to restore the normal number of spermatozoa and to eliminate the high proportion of immature sperm that showed up after frequent matings. Polovcova and Nagaev (1929) did not agree that sexual excitement in the stallion stimulates sperm production. Marked individual differences in spermatogenetic capacity were noted by Voloskov (1935), some stallions being able to yield sperm of high quality with 2 matings daily, while others needed a day of rest at varying intervals. Neumann and Salzman (1937) stated that the stallion could perform 2 and, in exceptional cases, 3 matings daily. Lambert and McKenzie observed that for many mature stallions it would appear that from 2 to 5 matings on some days can be made without lowering the average level of fertility, but that good management would seem to call for lighter use following days with heavy breeding schedules.

According to Polovcova prolonged sexual rest has a detrimental effect on spermatozoa ; motility and morphology are below normal in the first ejaculates.

Day (1940*b*) noted that the interval between services did not affect the quantity or density of the sperm in those horses where the interval in most cases varied between 6 hours and a week. However, in other horses, which were making more frequent services, five and six times a day, a much lower density was noticed although the volume was good. Intervals of less than one hour usually resulted in lower density and volume.

Boar

McKenzie, Miller, and Bauguess (1938) found that the frequency of ejaculation is an important factor affecting volume of semen, sperm number, sperm morphology, and duration of motility. Repeated ejaculations at intervals of 24 hours or less reduced semen volume below 200 ml. ; reduced sperm concentration to 15,000 to 50,000 per mm.^{3} and total number of spermatozoa to 2 to 5×10^9; reduced the duration of motility to 1 to 3 days ; and increased the number of abnormal spermatozoa to more than 20%. Two of the 3 boars which were used at 12-hourly intervals temporarily lost their desire to mate after the 3rd or 4th collections. With ejaculation at intervals of 48 hours or longer, the semen volume remained above 200 ml., the number of spermatozoa above 100,000 per mm.^{3}, the total number of spermatozoa per ejaculate exceeded 20×10^9, the number of abnormal spermatozoa did not exceed 10%, and the sperm remained motile for 5 days or more.

Spermatozoa were ejaculated at the rate of approximately 20×10^9 per day over periods of 11 to 24 days by 4 boars used at different intervals. A fifth boar, which was more sexually active, had a higher rate of spermatogenesis and ejaculated sperm at the rate of 33×10^9 per day over a 14-day period.

Intensive ejaculations did not alter the relative numbers of different types of abnormalities (also observed by Rodolfo, 1934*a*), but during periods of extreme sexual activity sperm with the cytoplasmic cap appeared, the number varying with the individual and the degree of sexual activity. Chemical analyses did not show any significant differences between the composition of the initial ejaculate and subsequent ones. There were no consistent variations in the liquid-gelatinous material ratio of normal whole semen which could be attributed to the frequency of ejaculations. These observations therefore did not show any differences in the rate of exhaustion of the several glandular systems—with repeated collections volume suffered less than sperm numbers. In the first ejaculate from boars following a period of sexual inactivity (1) the number of abnormal spermatozoa approached or exceeded 20%, and (2) the number of sperm was greatest, although the volume of the ejaculate was not necessarily the greatest.

According to Rodolfo and Timofeeva (1932) boars cannot be used more than twice, preferably only once, in 24 hours because the large amount of semen ejaculated at one mating exhausts the supply. Later, Rodolfo (1934*a*) stated that there should not be more than one mating a day and even then there should be an interval of rest after every 2 days. This applies particularly to the yearling boar which probably should not be used oftener than every other day, if he is to be used for as long as 2 weeks ; otherwise the number of sperm decreases markedly and immature sperm begin to predominate.

McKenzie, Miller, and Bauguess stated " The large volume of semen, the extremely great number of sperm per ejaculate, the relatively long time required for ejaculation and the chemical composition of his semen give some indication of the heavy drain on the protein, mineral and energy supply of the boar during excessive sexual activity. Observations on the effects of frequent ejaculation on semen volume, sperm numbers, duration of sperm motility, and sperm morphology indicate that yearling boars should not be used more often than once in 24 hours, and that best results might be expected at 48-hour intervals if the breeding season is to extend over a period of two weeks or more."

Sexual Behaviour

Somewhat less attention has been paid to the sexual behaviour of males than to other phases of reproductive capacity. The subject is of considerable importance, for unless a male is keen and able to copulate promptly, delay and upset in the breeding programme will arise. The present discussion will be restricted to what appear to be the more important and practical points. (*See* Stone, 1939*a*, for a discussion on sex drive, Marshall, 1922, for an account of the mechanism of erection and ejaculation, and Gunn, 1936, for a study of erection and ejaculation in the ram.) Beach (1942) has recently provided a detailed critical review of the central nervous mechanisms involved in the reproductive behaviour of vertebrates.

The sexual behaviour of an adult male with a fully receptive female covers all stages of sexual activity from normal copulation and ejaculation to complete lack of interest in the female. The different types and degrees of sexual activity may be represented on the following lines : (1) normal copulation and ejaculation, (2) mounts female, complete erection, intromission, but fails to ejaculate, (3) mounts female, failure of intromission, (4) mounts female, no erection, (5) failure to mount female, but licks vulvar region and hind quarters, (6) no interest other than standing near female and following her when she moves, (7) fails to approach female or moves off when placed with her.

Sexual behaviour in this context consists of the mating desire (libido, sex drive) and the ability to copulate. It is somewhat difficult to distinguish clearly differences in sexual activity due to mating desire on the one hand and mating ability on the other, as Lagerlöf (1934) has noted in the bull, particularly when the latter is not affected by any obvious incapacitating cause. Mating desire and mating capacity may vary quite independently of each other in the bull and ram (personal observations) but it is not unlikely that a direct association exists between the two, due to some common underlying cause. McKenzie and Berliner (1937) have observed in rams a period of increased mating ability as well as mating desire from September to December. Their observations suggest that breeding desire did not change markedly throughout the year, but that during February, March, and April the rams apparently did not complete copulation as readily as at other times. In this period erection of the penis seemed lacking or imperfect, resulting in failure of intromission, but at the same

time most of the rams continued to mount the ewes in attempts to serve them so that with failure of gratification the number of attempts to copulate increased; this should not be interpreted as increased mating desire.

Stone (1939*a*) has examined the intensity of sexual drive in laboratory animals. He observed that different animals of the same species varied greatly in sexual activity; with rats the numbers of copulations and ejaculations performed in a given time are variable for individuals. The numbers of ejaculations were only about 10% of the copulations but there was good correlation between frequency of intromission and frequency of ejaculation. Carpenter (1933) noted that a strong copulatory drive is associated with immediate copulation and a weak drive with delayed copulatory response.

Sexual behaviour can be measured in the following ways: (1) by allowing the male so many opportunities for copulation and ejaculation and stating his performance as a percentage of the possible, (2) by observing the number of completed copulations and uncompleted attempts at copulation in a given time period, as McKenzie and Berliner have done in the ram, and (3) by observing the time between the beginning of the test and the first copulation (provocative time, Carpenter, 1933).

Sexual behaviour is influenced by both internal and external factors. The internal factors include physiological, psychological, and physical conditions, and the external factors comprise climatic and nutritional conditions, disease, and, according to Macirone and Walton (1938), the suitability of the sexual object.

Sexual behaviour is influenced by endocrine factors. Castration of young males causes total or almost total absence of sexual responses and in adult males sexual aggressiveness gradually wanes after castration. In boars (which had learned to copulate at more or less regular intervals prior to the castration operation) McKenzie, Miller, and Bauguess noted that castration had little effect on libido, as judged by the collection of semen from animals 4 to 16 days after castration. Androgens are concerned with the growth of the male reproductive organs and with erection. They also have a marked effect on libido. Administration of testosterone acetate or propionate to immature rodents and monkeys, and to castrated and hypophysectomised rodents stimulated repeated erection (Hamilton, 1937). In rats, Stone (1939*b*) has shown that sexual activity is restored by testosterone propionate injections; in all rats the ejaculatory reflex was slower in returning than the copulatory response and was the first to go when treatment was stopped. Extinction of sex desire, which is rapid and sometimes almost instantaneous following removal of the pituitary, is restored by pituitary extracts, and as there is some evidence that the substance responsible for this effect is not identical with gonadotropic hormones the name " erogenic hormone " has been suggested for it (Lane-Roberts *et al.*, 1939).* McKenzie, Miller, and Bauguess noted that removal of the seminal vesicles, Cowper's glands and two-thirds of the prostate, and vasectomy did not reduce libido in the boar.

Stone (1942) found that male rats kept at a constant weight from 30 to 50 days of age showed a mean retardation of 10 days in initial appearance of copulation. This retardation was reduced to 3·3 days by daily injections of 0·25 mg. of testosterone propionate.

Once reproductive power has been attained, psychological factors, in the absence of testicular secretion, are apparently fairly successful in permitting mating, for a time at least. Psychological factors are of considerable importance in stimulating and maintaining good sexual behaviour. Males can be trained fairly easily to mount females out of heat and even dummies. According to Rodolfo (1934*b*) sexual attraction plays little part in the mating behaviour of the boar, and this animal is easily stimulated by the presence of a dummy sow. In the rabbit Macirone and Walton (1938) have found that completion of the sexual pattern depends on 2 supplementary factors, (1) the sexual drive shown by the male, and (2) the suitability of the sexual object. A strong male requires only the minimum of suitability of the sexual object to produce ejaculation, while a weak male may require maximum suitability (*i.e.* oestrous doe). The essential factor with a strong male is the sexual drive, which when a suitable object is provided, finds its expression in a complete sexual pattern. But it must be noted that a weak male is not merely inhibited by lack of suitability of the sexual object, but will in many cases not attempt to mount the dummy. With an oestrous doe, however, it will immediately show considerable activity and ultimately mate, indicating that some characteristic of the normal female apparently stimulates the male to increased sexual activity.

Training may be accomplished by accustoming the male to normal service in the same stall. Familiar surroundings, a regular daily routine, and handling the male in the same way each time are an essential part of good management. Slowness in service may be due to strange surroundings and to the nature of the control to which the animal is subjected (Marshall and Hammond, 1943).

* The relation of thyroid activity to libido has already been mentioned (*see* p. 28).

Travelling long distances may have a similar effect. Often bulls and stallions refuse to serve females on heat in unfamiliar surroundings or females of some specific colour (Milovanov, 1934; Lagerlöf, 1935). Milovanov cites experiments of Nagaev on rams, confirmed by McKenzie and Berliner, which show that the training of the male may interfere with his normal mating action and even inhibit it (conditioned reflex block). A ram was led for several days to a ewe in oestrus but separated from her by a gate so that he could not reach her; after a time, even when the gate was removed he did not attempt to reach her. McKenzie and Berliner believed that this might explain the behaviour of mature males during the anoestrous season—when they know that females not on heat will not let them mount they make no more attempts to do so. Also males get sexually excited when led towards the place where they are accustomed to serve females. Young bulls may show no sex desire when first put into service, probably because they are ignorant of what is expected of them (Lagerlöf, 1936). Roux and Hoffman (1935) mentioned that at the beginning of the mating season certain rams will not mate when controlled service is practised. This condition is considered psychological rather than physiological and may be remedied by leaving the ram alone with an oestrous ewe for a few hours or overnight.

Over-exertion, or inability to copulate, or pain felt every time the bull mounts the cow, may cause absence of, or diminished, sexual desire. Mating ability or capacity to copulate is affected by many causes, among which are over-work or enervating food (Lagerlöf, 1934). With lack of exercise and too rich bulky food, the bull may grow fat, heavy and clumsy or big-bellied, whereby the markedly pendent or "pot" belly acts as a mechanical obstruction and causes the penis to be directed too low. Morbid changes in the skeleton, joints, muscles, sinews, hooves, or peritoneum may cause pain when the bull mounts, thus impeding or preventing the act.

W. L. Williams (1920) and others maintained that an acute infection of the testes and seminal vesicles may cause pain on service and hence incapacity to copulate, but this is not always the case (Lagerlöf, 1934; Anderson, 1939*b*). Anderson noted that a bull with chronic epididymitis was capable of normal vigorous service, but it is not known if such bulls eventually lose all desire to serve. Fifty-nine bulls out of a total of 250 refused to serve when tested. Three of the 59 bulls were unable to complete the act of erection, and the remaining 56 showed faulty sex impulse. Of the latter bulls 31 were affected in the testis and epididymis, but the remaining 25 showed no clinical abnormalities. Lagerlöf (1934) observed that of 161 bulls slaughtered as sterile in one year, 8·8% were removed for poor sex desire and 54% for inability to serve. One bull has been observed which was keen to serve when the penis was acutely inflamed, but could not do so because of inability to protrude the penis (Anderson, 1939*b*). Various degrees of semi-erection and protrusion of the penis and torsion of the penis have been seen in the bull, causing incapacity to copulate. According to Richter (1938) preputial catarrh due to vaginitis, trichomoniasis, etc., prevents service; frequently the bull is keen to serve, but because of pain cannot perform coitus. Chronic preputial catarrh causing thickening of the prepuce may restrict protrusion of the penis. Contraction of the retractor penis muscle has a similar effect (Götze, 1934).

Most observations indicate that mating behaviour and sperm production vary independently of each other. In the bull, willingness and ability to mate are not an indication of fertilising capacity (Lagerlöf, 1934; Anderson, 1939*b*). Anderson has noted that bulls of all grades of fertility exhibit great variation in their ability and desire to serve, and not uncommonly a completely sterile bull will be much keener and more capable of service than a highly fertile one. McKenzie and Berliner (1937) stated that mating desire is not completely reliable as an indication of the sperm-producing capacity of individual rams, or of the spermatogenetic activity during the different seasons of the year. Their data do, however, show a relationship between the two, for Shropshire rams with little sex interest during the spring months had in general the lowest total production of spermatozoa, and more copulations were completed during periods of high fertility. Alifanov (1933-34) has also observed a correlation between sex interest and sperm production in the dog. It would seem probable that sexual behaviour and sperm production are influenced by a common underlying factor or factors. McKenzie and Berliner observed one sterile ram which showed a very marked sex interest. They found that good rams will continue to copulate when after frequent matings their sperm supply is temporarily exhausted; on the other hand, some rams after several services stop mounting the ewes, even though their last ejaculate still contained a normal number of spermatozoa. Similar observations have been made by Gunn and co-workers, and by the writer.

Kirillov and Morozov (1933) have observed a beneficial effect on the quantity and quality of the ejaculate if the bull has spent some time getting ready to mount. The superiority of second over first ejaculates in bulls, when collected within a short time of each other, is probably associated to

some extent with sexual excitement and the efficiency of ejaculation. Also, in rams, McKenzie and Berliner have shown that the number of sperm is less in the first ejaculate of rams that show a strong sexual impulse and mount the ewe very quickly than it is in subsequent ejaculates.

CHAPTER 4. PHYSICO-CHEMICAL PROPERTIES OF SEMEN

In this monograph semen as ejaculated is referred to as " semen," and semen from which spermatozoa have been removed by centrifuging or other physical techniques is called " seminal fluid " or " semen plasma." The word " sperm " has been used by Russian workers to designate whole semen as ejaculated, but American workers have restricted its use to a contraction of the word spermatozoa and it is in this sense that it is used here. Semen is composed of the spermatozoa themselves and the secretions of the accessory glands, and a distinction must be made, as far as possible, between the physico-chemical properties of the spermatozoa and those of the accessory secretions.

Osmotic Pressure

Determinations of the osmotic pressure in terms of the depression of the freezing point ($\triangle$) have been given by Roemmele (1927), Milovanov (1934), and Bernstein and Šergin (1936). Roemmele found an average of 0·62 and a range of from 0·54 to 0·73° C. for bull semen. The $\triangle$ of stallion semen is given by Ivanov (*see* Hartman, 1939) as 0·56° C., which is slightly lower than that given by Bernstein and Šergin (1936). Their data are as follows :—

Depression in ° C.	Bull.	Ram.	Stallion.	Boar.	Rabbit.
Average	0·609	0·641	0·595	0·616	0·574
Range	0·53-0·65	0·55-0·70	0·58-0·62	0·59-0·63	0·55-0·59

They obtained a $\triangle$ of 0·641° C. for the seminal vesicle secretion of the boar. On the whole the $\triangle$ of semen was found by them to be very similar to that of blood serum. Contrary to the findings of Roemmele these workers noted a very small range of $\triangle$.

The following data from Milovanov show the isotonic concentration of glucose from cryoscopic data :—

	M.	%.
Bull	0·357	6·4
Ram	0·335	6·0
Stallion	0·302	5·4

These figures are equivalent to a $\triangle$ of 0·66, 0·619, and 0·558 for bull, ram, and stallion semen respectively. For the bull the figure is above the range of Bernstein and Šergin and within that of Roemmele ; for the ram it is within and for the stallion below the range of Bernstein and Šergin.

Buffering Capacity

Šergin (1935) found that the buffering capacity of semen is lower than that of blood. In the semen of the bull and ram it is 2 to 3 times greater than in that of the boar, stallion, and rabbit. It is insufficient for maintaining the *p*H during storage. Stored semen of the boar and stallion showed a considerable change of buffering capacity within a *p*H range of 6 to 8, which suggests a destruction of carbonate buffer.

The neutralisation and buffer coefficient curves were very similar in four bulls studied by Smith and Asdell (1940). The buffer coefficient curves indicated that bull semen is relatively well buffered in the regions of *p*H 4 to 5·5 and 9 to 10 and relatively poorly buffered in the region of *p*H 6 to 9. The semen was more highly buffered on the acid side than on the alkaline. Blood serum was very similar to bull semen except that serum had a region of relatively high buffer capacity at about 6·5 to 7·0. On the whole, bull semen was as good a buffer as serum, if not better, over the *p*H range of 4 to 10·5, although in the limited range nearer neutrality, blood serum was a stronger buffer. Curves from seminal fluid were very similar to semen curves, which suggests that seminal fluid contributes much to the buffering capacity of semen. It was thus shown that bull semen is well buffered and will maintain favourable *p*H in spite of addition of considerable amounts of organic acids. The position of high buffering capacity near *p*H 5 probably explains, it is stated, why stored semen becomes more acid until a *p*H of about 5 is reached, following which the *p*H remains stationary. (*See also* p. 137.)

According to Dubinčik (1934) the addition of lactic acid to human semen caused a much greater shift towards acidity than did hydrochloric acid; apparently the buffering capacity was greater with mineral acids.

Electric Charge

The following information is taken from Milovanov (1934). Sperm from the rete testis, in an electric field, move slowly, partly towards the cathode, partly towards the anode and easily change their charge if the medium is modified. Sperm from the tail of the epididymis of the bull and the goat always move towards the anode, *i.e.* they have a negative charge, the magnitude of which is greater than in sperm from the rete testis. The mammalian spermatozoon is said to have a small strongly negative region at the anterior end, while the remaining portion and the tail have a positive surface layer. The nucleus and membrane of the ovum have a positive charge, and the behaviour of the spermatozoon in the presence of the ovum, the attraction of the head and repulsion of the tail, are explained by electrostatic attraction and repulsion.

The magnitude and sign of the electric charge may alter with change of the medium, the principal factors affecting it being the hydrogen-ion concentration and the cations of metals. The *p*H at which the charge is altered (about 3·0 for sperm of the sea urchin and frog) is toxic for sperm and they lose their motility. An alteration in the charge of rabbit sperm at *p*H 4·75 to 4·80, and of stallion sperm at *p*H 5 to 6, has been obtained by Schröder (1932), without, however, loss of motility. This divergence from the results of other workers may be explained, stated Milovanov, by the sensitising effect of glycocoll, which was present in the buffer solution used by Schröder. This sensitisation is accomplished by substances that easily alter their charge upon the cells which absorb them.

The most active cations are the divalent (Ca, Mg) and ter- and quadrivalent cations, whose positive charges are capable not only of discharging, but of altering the charge of the sperm, while univalent cations (Na, K) usually merely eliminate the negative charge, but do not recharge the sperm. Removal of the charge, as a rule, leads to agglutination, which may be observed when semen is diluted with salt solution such as NaCl and Na_2SO_4. Agglutination has a great practical importance because agglutinated sperm lose their fertilising ability. The substitution of salt by non-electrolytes (*e.g.* glycocoll) prevents removal of the charge and therefore agglutination.

Electro-conductivity

Bernstein and Šergin (1936) gave the following particulars of the electro-conductivity of semen :—

Electro-conductivity at 25° in reciprocal ohms $\times 10^4$.	Bull.	Ram.	Stallion.	Boar.	Rabbit.
Average . . .	104·57	63·2	122·97	128·95	93·78
Range	89·5-116·3	48·52-80·5	111·31-129·53	123·3-134·6	85·54-101·4

The figures are similar to those for blood serum in the stallion and the boar, and differ greatly for the bull, ram, and rabbit.

The data of Pojarkov (*see* Milovanov, 1934) on the semen of the horse and dog, which is particularly rich in accessory secretions, show the following figures for electro-conductivity: horse

$116{\cdot}1 \times 10^4$, dog $145{\cdot}2 \times 10^4$. Popova (*ibid.*) found that the majority of electrolytes of any accessory secretions belong to the chloride salts of sodium, calcium, and potassium. The amount of Cl in the semen of the stallion is on the average 479 mg.%, while in that of the ram, which is very poor in accessory secretions, it is only 46 mg.% ; this ten-fold rise corresponds to the ten-fold rise of electro-conductivity of stallion semen. It is thought that the low electro-conductivity of the epididymal contents in the bull may be due to low epididymal content of NaCl.

Inorganic Constituents

Bernstein (1933*b*) made chemical determinations on the semen of the bull, stallion, dog, and man. There was considerable variation in the sodium content in samples from different individuals and in different samples from the same individuals. On the whole, semen plasma gave slightly higher figures (35·9-230 mg.%) than whole semen (57·5-201·2 mg.%). Bull semen plasma contained 109·7 mg.% compared with 320 mg.% in blood serum. The range of variation in the potassium content was considerable. In ejaculates with a high content, 250-400 mg.%, the concentration of potassium was somewhat higher in plasma than in whole semen, whereas when the content is lower, whole semen was relatively richer in potassium than the plasma. Bull semen plasma contained 255·8 mg.% potassium compared with 21·7 mg.% in blood serum. The Na/K ratio in bull semen plasma was 0·4 and in blood serum 15·2. As regards calcium content there was no marked difference between semen plasma (17·2-43·1 mg.%) and whole semen (24·1-45·4 mg.%). Bull semen plasma contained about 5 mg.% magnesium and whole semen 14·2-21·4 mg.%. Whole semen of the dog contained 2·8 mg.%. The chloride content was more stable than that of the cations studied ; bull semen approaches that of the stallion and differs greatly from the dog, as the following data show :—

Stallion . . .	86·0 to 443·0 mg. per 100 ml. semen		
Bull . . .	309·5 to 433·0	,, ,, ,,	or semen plasma
Dog . . .	620·0 to 657·0	,, ,, ,,	

The sulphate content of semen of different species is roughly similar, about 2-4 mg. per 100 ml. Semen is very rich in both inorganic and organic phosphates ; Bernstein (1933*b*) gives the following figures (*see also* Iljasov, 1933) :—

	Inorganic Phosphates (in mg.% P_2O_5).		Total Phosphates (in mg.% P_2O_5).
	Average	Range.	
Bull	31·4	13·6-51·2	127·0
Stallion	39·0	32·0-52·0	43·2
Dog	45·5	37·0-54·0	64·0

With regard to the concentration and interrelations of cations such as Na, K, and Ca, semen and its plasma differ considerably from blood serum, which is particularly rich in Na, the amount of Na being 15 times or more greater than that of K, whereas in semen plasma the K content is very great. The Ca content of semen plasma is 3-4 times greater, namely 17-43 mg.% as compared with 8 mg.% in blood serum.

These facts suggest, in Bernstein's view, that there exists in the male genital tract a complex polyfunctional glandular epithelium which is responsible for the active and selective secretion of the various mineral compounds of seminal fluid. The liquid medium which surrounds the spermatozoa in the genital tract, and after ejaculation, markedly differs from various physiological solutions in its composition and in the interrelations of the most important cations. This conclusion is based not only on the chemical composition of semen but also on the marked variation in its composition. It would appear that sperm possess a high degree of resistance to variation in content of certain mineral compounds in the medium.

According to Šergin (1933) the amount of ammonia in the fresh semen of the stallion and the

bull is very slight and in some cases cannot be detected (maximum 2·71 and 1·22 mg.% respectively). Variation among individuals of the same species is fairly great, possibly due to varying degrees of freshness of semen or more probably to differences between ejaculates, since semen always contains a variable proportion of dead, injured, or degenerated sperm which would probably, it is considered, lead to a greater accumulation of ammonia. There is an accumulation of ammonia when semen is stored outside the body ; it is intensified at high temperatures and very much depressed at low temperatures. It would seem that the ammonia in fresh semen, like that formed on storage, is the product of biochemical changes that occur in the protoplasm of spermatozoa after death.

Boar.—Nesmejanova (1936) made chemical analyses of the 1st, 2nd, and 3rd fractions of ejaculated boar semen and of the secretions of the epididymis and seminal vesicles collected from slaughtered animals. The first fraction was a clear liquid fluid with a small quantity of formative elements, chiefly epithelial cells and fat droplets, the second was the sperm-containing fraction, and the third fraction, containing a few spermatozoa, was cloudy and thick. The thick tapioca-like material from Cowper's glands was not included in the analyses. The chemical determinations were made on centrifuged material. Her results are shown below (all values in mg. %) :—

	K.	Na.	Ca.
Semen, 1st fraction	54·19	311·82	5·77
2nd fraction	99·76	284·5	8·88
3rd fraction	56·06	305·2	5·77
Epididymal secretion	245·8	97·02	9·17
Seminal vesicle	167·0	62·7	9·44

Chemical analysis by McKenzie, Miller, and Bauguess (1938) of the liquid and gelatinous portions of normal whole semen showed that except for the gelatin portion having somewhat more sodium and calcium, there was no significant difference in the composition of the two materials. The following table gives the chemical analysis of fractionated whole semen from normal boars in mg. per 100 g. wet weight :—

Constituent.	Pre-Sperm Fraction.	Sperm Containing Fraction.	Post-Sperm Fraction.
Weight (g.)	15·1-67·4 31	21·8-140 66	26·8-244 126
Dry matter (% of wet weight)	4·90-6·14 5·06	4·16-6·17 5·22	4·14-6·43 4·95
Sodium	558-892 762	522-103 763	554-104 727
Potassium	128-285 177	123-376 243	167-314 237
Calcium	6·1-9·3 7·8	5·1-8·9 7·3	6·2-9·6 7·4
Magnesium	7·3-13·0 10·1	7·2-17·1 12·2	8·2-13·8 11·1
Phosphorus	4·6-13·0 9·8	5·8-14·4 9·9	6·2-12·7 9·8
Chlorides (as NaCl)	448-601 502	448-637 535	400-742 515

Note.—The figures in the upper rows give the range, the value below is that of the mean.

Chemical data relating to the boar, determined by the same workers, are shown in the accompanying tables.

The Chemical Composition of Boar Semen:

(from McKenzie, Miller, and Bauguess, 1938)

(mg. per 100 g. wet weight)

Nature of Material.	No. of Animals Involved.	No. of Samples Analysed (duplicated).	*p*H	Dry Matter, Per Cent. of Wet Weight.	Inorganic Constituents.					
					Sodium.	Potassium.	Calcium.	Magnesium.	Phosphorus	Chlorides. (as NaCl).
Blood serum	4	4	7·6-7·7 7·6*	8·1-8·2 8·14	186-227 203·0	22·7-25·2 23·5	9·7-11·5 10·3	2·0-4·5 2·9	6·7-8·7 7·9	495-600 551·0
Blood plasma	4	7	7·5-7·6 7·5*	8·4-9·2 8·7	... ...	32·5-38·2 36·1	... ...	... ...	8·3-9·3 8·6	580-622 593·0
Seminal vesicle fluid	5	9	6·4-6·8 6·7*	15·8-18·4 16·3	300-606 398·6	1043-1471 1244·8	7·2-8·7 7·8	38·1-44·7 39·7	39·0-51·0 43·1	15·0-60·0 32·4
Cowper's gland material	5	8	7·2-7·3 7·2*	14·5-18·3 15·8	857-1330 1094·6	422·0-557·0 493·1	27·1-38·0 30·1	128·0-166·0 140·6	8·9-11·8 9·8	377-435 408·6
Epididymal fluid	3	3	6·7-6·9 6·9*	10·8-14·2 13·2	277-688 482·0	249·0-399·0 324·0	... ...	... ...	158·0-280·0 219·0	158-480 356·0
Normal whole semen	7	19	7·3-7·8 7·5*	2·2-6·2 4·6	280-837 646·1	83·0-382·0 243·0	2·3-6·6 5·21	5·2-14·5 10·7	3·7-16·9 8·4	423-701 542·1
Semen from boar without seminal vesicles	1	3	7·6-7·8 7·7*	2·8-3·4 3·1	267-327 296·0	59·0-68·0 63·0	6·7-7·8 7·3	1·1-2·5 1·6	1·7-2·8 2·3	586-617 597·0
Semen from boars without Cowper's glands	2	8	7·2-7·4 7·3*	3·8-7·4 4·8	286-544 400·0	75·0-144·0 100·0	3·9-6·5 5·4	5·4-24·2 10·7	12·7-56·3 29·0	485-723 565·0
Semen from boars without seminal vesicles or Cowper's glands	3	11	7·4-7·8 7·6*	1·2-2·4 1·6	244-364 307	20·0-101·0 57·3	1·8-4·6 3·1	0·7-1·7 1·6	9·1-18·8 12·9	622-808 714·3
Semen from vasectomised boars without seminal vesicles or Cowper's glands	4	16	7·5-8·5 8·0*	0·9-2·3 1·2	24·77 41·5	15·0-68·0 28·6	1·1-3·5 1·8	0·5-0·5 0·5	0·1-0·1 0·1	624-818 753·3

Nature of Material.	Organic Constituents.				
	Glucose.	Urea N.	Apparent Creatinine.	Total Nitrogen.	Protein Equivalent.
Blood serum	80·7-91·0 85·4	12·2-14·2 13·5	2·4-2·4 2·4	957-1002 982·0	5984-6262 6135·0
Blood plasma	80·0-89·0 83·9	16·2-18·0 17·0	2·4-2·4 2·4	950-1143 1010·0	5941-7144 6315·0
Seminal vesicle fluid	243·0-266·0 257·0	... ...	4·9-5·6 5·3	1470-1512 1488·0	9187-9450 9302·0
Cowper's gland material	... ...	... ...	... ...	1238-1296 1275·0	7737-8100 7969·0
Epididymal fluid	... ...	... ...	... 1·4	... 1606·0	... 10038·0
Normal whole semen	12·9-56·0 36·6	... 0·0	0·1-0·3 0·3	334-765 613·0	2088-4785 3831·0
Semen from boar without seminal vesicles	0·0 0·0	0·0 0·0	0·4-0·4 0·4	215-239 225·0	1344-1499 1408·0
Semen from boars without Cowper's glands	41·8-111·0 60·8	0·0 0·0	0·7-1·2 0·9	401-600 466·0	2500-2650 2914·0
Semen from boars without seminal vesicles or Cowper's glands	0·0 0·0	0·0 0·0	0·3-0·4 0·3	91-188 133·2	569-1175 832·5
Semen from vasectomised boars without seminal vesicles or Cowper's glands	0·0 0·0	0·0 0·0	0·2-0·4 0·3	55-109 73·7	344-681 461·0

* Median.

Note.—The numbers appearing in the upper rows give the range, while the value below is that of the mean.

Most of the potassium and phosphorus comes from the seminal vesicles, most of the sodium, calcium, and magnesium from Cowper's glands, and most of the chlorides from the prostatic and urethral secretions.

Nesmejanova's results were adversely criticised by McKenzie and co-workers, whose determinations failed to support Nesmejanova's views on the similarity between the composition of semen and blood serum, especially with regard to electrolytes, and her suggestion that the great dilution of semen was produced by material similar in composition to blood serum. Their results show that blood serum contains 7 times as much dry matter, 5 times as much sodium, 6 times as much calcium, 6 times as much magnesium, and 79 times as much phosphorus, as prostatic and urethral secretions. Chlorides were materially higher in prostatic and urethral fluids than in blood serum. In reply to the criticism of McKenzie and collaborators, Nesmejanova (1940) has recalculated their data on the amounts of Na, K, and Ca in isotonic amounts of NaCl; the total quantity of electrolytes is equivalent to over 2 g. per 100 cm.^{3} of fluid, *i.e.* the osmotic pressure is more than twice the normal (15·4 *v.* 7·2 atmospheres). The electrolyte concentration of Cowper's gland secretions is nearly 4 times, and that of seminal vesicle secretion 3 times, the normal. Semen free of accessory secretions had an osmotic pressure corresponding to the normal. When Nesmejanova's own data were similarly recalculated, the osmotic pressure was found to range about normality ($\equiv$ 0·85-0·87 g. NaCl per 100 cm.^{3}); that of the accessory secretions was lower than that of sperm, which, it is stated, agrees with other data. In order to eliminate the effect of preliminary treatment, comparative analysis was made by two methods, protein sedimentation as used before, and wet incineration as used by McKenzie and co-workers; no significant difference was observed, and figures obtained in both cases are stated to confirm the earlier results of Nesmejanova.

Organic Constituents

The initial sugar and lactic acid content of the semen of different animals is shown in the accompanying table.

	Sugar (as glucose).		Lactic Acid.		Author.
	Average.	Range.	Average.	Range.	
Ram . .	179	123-270	126	45·5-187	Šergin, 1937
	516·3	217-744	120	119 & 121	Moore and Mayer, 1941
Bull . .	334	281-495	63	48·4-75·8	Šergin, 1937
	300	100-400			Bernstein, 1933*b*
				40-50	Bernstein and Slovohotov, 1933
Stallion . .	82	67-116			Bernstein, 1933*b*
	3·5	0-8	26·2	4·6-71·5	Šergin, 1937
		4-18			Davis and Cole, 1939
Dog . .	116	80-156			Bernstein, 1933*b*

Values expressed as mg. per 100 ml.

Moore and Mayer (1941) used on ram semen the Shaffer-Hartman-Somogyi method which gives a filtrate containing practically no reducing substances other than glucose. The interval between ejaculation and protein precipitation was in no case more than 2 minutes. The values for the initial sugar concentration of 7 rams during the natural breeding seasons show the wide variations that occur. Thirty-three values for the semen of an individual ram gave a mean of 682·8 mg. per 100 ml. with a standard deviation of 154·4 and a coefficient of variation of 22·6%. A second ejaculate collected usually 5 to 15 minutes after the first had invariably a higher sugar content. Yeast fermentation of samples of ram semen gave a value of 42-45 mg. per 100 ml. for the non-sugar reducing substances in ram semen, which corresponded to the quantity of non-sugar reducing substance remaining in experiments after the removal of sugar by glycolysis.

The ejaculates of a vasectomised ram gave initial sugar values approximately 2·5 times those of normal semen. The coefficient of variation of " vasectomy semen " was 12%, compared with 21% for normal semen. From this lowered variability, combined with the obviously smaller volume and the increase in sugar concentration to more than double the pre-operation level, it is concluded that more than half the volume of ram semen comes from the epididymis and that the sugar is in the main a product of the accessory genital organs, *i.e.* seminal vesicles, Cowper's glands, and prostate.

Bernstein (1933*b*), using the Hagedorn-Jensen method which determines not only glucose, but the total amount of all reducing substances, found an average glucose content of about 300 mg.% (range from less than 100 to 400 mg.%) on 92 samples of fresh bull semen. It was found that sugar is present in both the spermatozoa and the semen plasma ; the glucose content in the latter is very variable and that of the spermatozoa somewhat more stable. The calculation to show that spermatozoa themselves contain glucose was based on the glucose content of whole semen and seminal fluid and on the packed cell volume of spermatozoa. The smaller the number of spermatozoa in semen the lower was the glucose content.

Boar and stallion sperm taken from the ducts before ejaculation contain small amounts of lactic acid ; this is attributed to a slow process of glycolysis during anoxybiosis and partly to the activity of the duct tissues (Šergin, 1935). Davis and Cole (1939) stated that stallion semen differs from that of other animals in its low glucose content (4 to 18 mg.%) ; the lactic acid content is also low in semen which has aged.

The organic constituents of boar semen are shown in the table from McKenzie, Miller, and Bauguess (1938) (*see* p. 56). Urea was not detected in any seminal fluids by the method used, and creatinine was present in very small amounts. Total organic nitrogen was present in rather large amounts in all the seminal fluids, except the prostatic and urethral secretions. The highest concentration of nitrogen was found in epididymal fluid, followed by seminal vesicle fluid and Cowper's gland material in that order.

Iljasov (1933) determined the creatinine and creatine content of semen (in mg. % creatinine) as shown below :—

Animal.	Mean Creatinine.	Mean Creatine.	Mean Creatinine/ Creatine Ratio.
Stallion	3·7	6·2	0·59
Bull	12·1	3·0	6·41

Creatine is predominant in the semen of the stallion, but that of the bull contains less creatine than creatinine. Whole semen is much richer in creatinine than semen plasma, and spermatozoa contain more creatinine than creatine ; this would explain the difference in content of these substances in the semen of the bull and stallion, since bull semen has a much greater concentration of spermatozoa. When semen was stored outside the body it was found that the amount of phosphagen decreased. In many cases a close relation was evident between the duration of motility of sperm and the decomposition of creatine phosphoric acid.

The citric acid content was investigated by Scherstén (1936). In the seminal vesicles it varied with age, being less in younger animals, as confirmed by determinations in the guinea-pig and human. It was decided that the citric acid buffer system in semen is not of biological importance. A study of the relation between citrate content and calcium and phosphorus indicated the importance of citrate in keeping part of the calcium content of semen in solution. The addition of citrate to Ringer-Tyrode prolonged the life of sperm of the bull, ram, and boar.

In observations on two halfbred Brabançon stallions, Voloskova (1938) found lecithin in both spermatozoa and seminal fluid, and cholesterol only in the former. It is stated that the cholesterol content of semen may thus serve as an index of its density. After intense sexual activity, the lipoid level of semen decreased and a 3 to 6 days' interval was required to restore it. With moderate sexual activity, survival was longest when the cholesterol : lecithin ratio equalled 1.

The average content of CO_2 per 100 ml. semen is : boar 50 ml., stallion 24 ml., and bull and ram 16 ml. (Šergin, 1935).

Phillips (1940) gave the normal value for the ascorbic acid content per 100 ml. of semen and blood plasma of bulls as from 3 to 7 and 0·2 to 0·4 mg. respectively. In man and the guinea-pig the values averaged, for whole semen, 12·8 and 8·2 mg. per 100 ml. respectively, which is higher than the blood plasma value, due to the high content in the seminal vesicles. In the dog, on the other hand, which has no seminal vesicles, the semen has the same content as the blood plasma (Berg, Huggins, and Hedges, 1941).

From determinations on 43 ejaculates from 10 human subjects, Gutman and Gutman (1941) found values from 700±150 to 3700±500 U acid phosphatase per ml. of seminal fluid. The enzyme levels in the several ejaculates of any one individual were relatively constant. They also examined samples from 24 sterile men and in only one was the acid phosphatase activity low (33 U per ml. of seminal fluid).

Lardy and Phillips (1941*b*) found an active succinic dehydrogenase in bull semen. An alkaline phosphatase, whose activity was from 50 to 100 times as great as that present in blood plasma, was found in semen. The enzyme was found in both sperm and seminal fluid with by far the greater concentration per unit volume in the former.

Riboflavin was found in both sperm and seminal fluid. Flavin-containing enzymes are known to function in the hydrogen transport mechanisms of other tissues and it is probable, state Lardy and Phillips, that similar mechanisms exist in spermatozoa.

Zittle and Zitin (1942) demonstrated the presence of the cytochrome system in bull sperm, the cytochrome oxidase content per mg. of tissue being about the same as that found in rat skeletal muscle ; they also confirmed the presence of the succinic dehydrogenase. The greatest cytochrome oxidase activity was found in the mid-pieces and tails, the heads being metabolically inert in the respiratory sense. MacLeod (1943*a*) considers that their statement that the sperm head is probably a metabolically inert carrier of the chromosomes should be taken with reserve since bull sperm have a high aerobic and anaerobic glycolysis which possibly could derive from tissue devoid of haemin iron.

Acetyl choline or some similar substance is present in human seminal fluid (Cockrill, Miller, and Kurzrok, 1935). When present in unduly large amount it causes contraction of human uterine muscle and it is probably also responsible for the normal relaxation of uterine muscle. Its pharmacological activity is considered of importance in the physiology of sperm entry into the uterus.

The tubal egg of the rat, surrounded by the cumulus cells and corona radiata, is embedded in a transparent, viscous fluid or gel (Long, 1912) and the removal of this substance and the consequent disintegration of the cell mass is necessary to enable the sperm to penetrate the egg. It has been suggested that the dispersal is brought about by a heat-labile substance produced by the sperm (Pincus and Enzmann, 1935). An enzyme, hyaluronidase, is associated with sperm and McClean and Rowlands (1942) have shown that this enzyme causes liquefaction of the gel. Hyaluronidase belongs to a group of enzymes which hydrolyse hyaluronic acid and the evidence suggests that the gel surrounding the eggs in the Fallopian tubes contains hyaluronic acid.

Zittle and O'Dell (1941*a* and *b*) studied, in bull spermatozoa, the lipid, sulphur, cystine, nitrogen, phosphorus and nucleic acid content of whole sperm and of the parts obtained by physical means. The lipid, P, and N content of the heads and tails were 6-7 and 23, 4·0 and 0·5, 18·5 and 13·6% respectively. All the phosphorus was present as thymo-nucleic acid and $\frac{2}{3}$rds of the sulphur (1·5-1·8%) was accounted for as cystine-cysteine. The presence of a keratin-like protein was indicated. The S and P contents of guinea-pig and human sperm were approximately equal to those of bull sperm. Methionine and cystine contents of spermatozoa were : whole 1·8 and 4·0%, heads 1·0 and 4·0%, midparts 2·2 and 4·4%, and tails 3·4-4·0 and 2·4-3·3% respectively. These authors state that there is more than one S-containing protein in spermatozoa.

Green (1940*a*) observed that ram sperm contained a large amount of sulphur as cystine, and the evidence also suggests the presence of a keratin-like protein.

Huggins, Scott, and Heinen (1942) reported that the proteins of human semen are largely proteoses (60%). The average globulin content found was 1·2 g.%, accounting for approximately 30% of the total seminal proteins. Gray and Huggins (1942) observed four protein fractions electrophoretically in normal and pathological human semen ; these had the same motility as albumin and alpha, beta, and gamma globulin found in blood serum and are presumed to be identical with these protein fractions. Ross, Moore, and Miller (1942) have given more complete electrophoretic and chemical data on the seminal proteins. They have chemically separated a glycoprotein in electrophoretically homogeneous state which contains 11% hexosamine and no uronic acid. Their figure for albumin is only about one-thirtieth of that given by Gray and Huggins.

The Contribution of the Different Accessory Glands to the Semen

The volume of the ejaculate will depend, among other things, on (1) the efficiency of the ejaculatory reflex, (2) the concentration of spermatozoa, and (3) the amount of the accessory secretions. Any condition, physiological or pathological, which affects these three factors may alter the volume of the ejaculate. Although the volume occupied by the sperm is a relatively small part of the total ejaculate, the bulk of which is formed by the accessory secretions, there is, in the bull, a direct correlation between the volume of the ejaculate and the concentration of spermatozoa, except when the ejaculate is unusually large, and then the concentration of spermatozoa is usually lower. In the bull the volume of the ejaculate may be unaltered in abnormal testicular conditions which cause sterility, but on the other hand with abnormal conditions of the accessory glands the volume may be reduced.

Boar.—The following account is taken from McKenzie, Miller, and Bauguess (1938). In the boar the rate of spermatogenesis, the extent and development of the accessory glands, and the volume of the ejaculate greatly exceed those of other farm animals. The seminal vesicles contribute grey, opaque, medium viscous fluid to the semen ; Cowper's glands, a thick, waxy material ; the prostate and urethral glands, a clear slightly viscous fluid, and the epididymis a milky white fluid.

From the knowledge of the nature of the pure glandular secretions of the male accessories, it is possible to obtain a reasonably accurate picture of the order in which the separate glandular systems make their contribution to the semen during the period of ejaculation. The thick jelly-like material in the initial fraction is apparently a mixture of Cowper's gland material and fluid from the urethral or prostate gland, for its high *p*H eliminates the seminal vesicle fluid, and it does not have the consistency of the tapioca-like material which appears later.

Following the initial discharge comes the high sperm-containing fluid from the epididymis. This is diluted with secretion from the prostate and urethral glands, plus some tapioca-like material. During this sperm phase the rate of ejaculation and volume discharge is usually greatest. A large volume continues to be discharged for a brief period, during which the sperm concentration decreases rapidly. The evidence that this large volume comes from the prostatic and urethral glands is based on its watery appearance, its high *p*H, and the fact that in operated animals which have only the prostate and urethral glands left, the maximum rate of discharge occurs at this same relative period in the ejaculate.

The greatest amounts of gelatin- or tapioca-like material appear immediately following the high sperm-containing fraction, and again near the end of the ejaculation. The necessity for both seminal vesicle and Cowper's gland for its formation is indicated by the absence of this material in semen from boars whose seminal vesicles and Cowper's gland have been removed. But a third substance from the urethral or prostatic glands may also be necessary, for the addition of fresh seminal vesicle fluid to fresh Cowper's gland material produced a gelatinous formation similar to, but not identical with, that of normal semen.

A method of calculating the relative volumes contributed to the ejaculation by the separate glandular systems is afforded by data on semen volume, sperm concentration, and chemical analyses of seminal fluid. The seminal vesicles contributed 26% of semen volume and the Cowper's glands 19%. Removal of both pairs of glands reduced semen volume 42%, and vasectomy further reduced it 2%. Therefore 56% (147 ml.) of the total semen volume consisted of prostate and urethral secretions, 42% (10 ml.) came from the seminal vesicles and Cowper's glands, and 2% (5·5 ml.) from the epididymides and testes.

Epididymal fluid collected directly from the epididymides of 7 boars, when slaughtered or castrated, contained 4·56 to 6·8 million sperm per mm.^{3} with an average of 5·8 million. With this number of spermatozoa per mm.^{3} in epididymal fluid, it would require 4·2 ml. of such fluid to supply the average number of sperm in one ejaculate from normal boars. With an average volume of 262 ml. the epididymal fluid must have been diluted 62 times, or expressed in another way, must have contributed 1·6% of the total volume of the semen.

Cellular debris was thrown down by centrifuging only in semen from boars whose testes, epididymides, and vasa deferentia were intact. The sediment/supernatant ratio affords a third way of calculating the volume of the contribution from this source. The sediment in epididymal fluid averaged 0·51 ml. The volume of sediment in the entire ejaculate divided by 0·51 gives the volume of the contribution from the testes and epididymides. The average of 27 collections of whole semen from 5 normal boars indicated 6·3% (range 2-10%) of the total volume of the ejaculate as coming from the testes and epididymides.

Another approach to the contribution from the various glands is afforded by the glucose content

of the seminal vesicle fluid and semen. The average glucose content of 9 samples of pure seminal vesicle fluid was 2·57 mg. per ml. Each unit of 2·57 mg. glucose in an ejaculate therefore represents 1 ml. of seminal vesicle fluid. Calculated on this basis, the average of 17 ejaculates from 5 normal boars gave 14·7% of the total volume contributed by the seminal vesicles and 85·3% from other sources.

In general, it is estimated that the seminal vesicles, Cowper's glands, prostate and urethral glands, and the epididymal fluid contribute respectively, 15 to 25%, 10 to 20%, 55 to 70%, and 2 to 5% of the semen volume. The large volume of secretion coming from the prostatic and urethral glands indicates their intense secretory activity. The liquid/gelatinous ratio is on the whole fairly constant, averaging about 70% liquid for the entire ejaculate.

Ram.—Gunn concluded that the great bulk of a normal ejaculate is composed of accessory secretions and that, though the spermatozoa may number 2500 millions per ml., they actually occupy very little space. Single and even double vasectomy had very little effect on the volume of the ejaculate. Semen collected from fistulae of the vasa deferentia usually formed less than one-tenth of the total material emitted through fistulae and penis combined ; and fluid from fistulae contained in addition to spermatozoa, and to any secretion from the testis, some secretion from the epididymis and proximal end of the vas deferens (Marshall, 1922). From a series of determinations on sugar concentration in the semen of the ram, Moore and Mayer (1941), using the artificial vagina, found that after vasectomy the sugar had increased to approximately 2·5 times the pre-operation level, whereas the volume of the semen was less than half that normally obtained from the ram before operation. From the changes in sugar concentration and in the volume of ram semen after vasectomy, and the proportion of the total semen volume occupied by the sperm cells, it is indicated that the sperm in the epididymides are suspended in a volume of fluid approximately equal to their own volume. At the time of ejaculation the suspension from the epididymides receives an addition of very nearly an equal volume of fluid from the accessory genital glands, *i.e.* normal ram semen is composed of $\frac{1}{4}$ sperm, $\frac{1}{4}$ epididymal secretion, and $\frac{1}{2}$ accessory gland secretion. The difference between these results and those of Gunn is probably due to the greater stimulation of the accessory glands by the electrical method, resulting in a larger ejaculate with a lower concentration of spermatozoa.

Physico-chemical Contributions.—In the boar the *p*H of the secretions from the seminal vesicles, Cowper's glands, epididymides, prostate and urethral glands is 6·7, 7·2, 6·9, and 8·0 respectively ; ejaculated semen is alkaline because of the relatively large volume of alkaline fluid contributed by the prostate and urethral glands (McKenzie *et al.*, 1938).

In the bull the *p*H of the testis is 6·7 and of the tail of the epididymis 6·1 (Lanz and Malyoth, 1928) or 6·7 (Becher, 1930). Dougherty and Ewalt (1941) found that the *p*H of fluid taken from the seminal vesicles, in abbatoir material, ranged from 5·65 to 6·43.

The *p*H of 8 samples of prostatic fluid from the dog varied from 5·29 to 6·16 (Huggins *et al.*, 1939). The tail of the epididymis of the rat (Lanz, 1929) and of the guinea-pig (Lanz and Malyoth, 1928) is acid. In the rat the seminal vesicle is acid and the prostate alkaline (Lanz).

According to Redenz (from Milovanov, 1934) the electro-conductivity of the fluid of the rete testis varied from 38×10^4 to 79×10^4, while the maximum observed for the contents of the tail of the epididymis was $10{\cdot}7\times10^4$.

The composition of the prostatic fluid in the dog is shown in the accompanying table from Huggins *et al.* (1939). The concentration of osmotically active substances was 335 mM per kg. of

(*Mean values are expressed per litre of fluid, with standard deviations*)

	No. of Fluids.	H_2O.	Cl.	Na.	K.	Ca.	NPN.	Total Protein.	Glucose.
		g.	mM.	mM.	mM.	mM.	g.	g.	mg.
Normal prostatic fluid	17	981±3	160±2·7	159±2·6	5·1±0·2	0·3	0·22	8·25	0·30
Castrate, injected with testosterone . .	17	984±3	162±3·6	160±3·3	5·2±0·2	0·3	0·31	6·14	0·75

water, of which the total base concentration consisted of 162 mM of sodium and 5·2 mM of potassium. Chloride concentration was 163 mM per kg. of water, thus accounting for most of the acid ions. The protein concentration was less than 1%.

In the boar the seminal vesicles contribute all the glucose, and most of the potassium, phosphorus, and total nitrogen in the semen. Cowper's glands contribute most of the sodium, calcium, magnesium, and also considerable nitrogen. The prostatic and urethral secretions are the source of most of the chlorides. There is a consistent reduction of sodium, potassium, calcium, and magnesium, and an equally persistent increase in chlorides in semen as the various glandular systems are eliminated by operation. The total contribution of any constituent from the testes and epididymides, because of their relatively small volume, is probably small, but the epididymal fluid is rich in phosphorus and total nitrogen (McKenzie *et al.*, 1938). In Cowper's gland material the Na : K ratio is about 6 times greater than that of seminal vesicle fluid. The K : Ca ratio of seminal vesicle fluid is about 10 times greater than that of Cowper's gland material, and the P : Cl ratio about 4 to 15 times greater. In the prostatic and urethral secretions the relative concentrations of the various inorganic constituents are quite unlike the corresponding values in blood serum and plasma (*see* Table p. 56).

In the ram, glucose is a product of the accessory glands, *i.e.* seminal vesicles, Cowper's glands and prostate (Moore and Mayer, 1941), and in man it comes from the seminal vesicles rather than from the prostate (Huggins and Johnson, 1933 ; Goldblatt, 1935). The sugar concentration of mixed human prostatic and urethral and seminal vesicle secretion was near that of human semen (McCarthy *et al.*, 1928). Bernstein (1937) divided the individual accessory glands into two groups : (*a*) sugar-secreting glands, which probably include the testes, epididymides and seminal vesicles, and (*b*) glands whose secretions contain no sugar, the prostate and bulbo-urethral glands.

In the dog an ascorbic acid content of semen greater than that of blood plasma seems to be associated with the seminal vesicles (Huggins *et al.*, 1939). According to Dougherty and Ewalt (1941) the ascorbic acid content of seminal vesicle fluid of the bull varies from 0·97 to 8·08 mg. per 100 ml.

Human prostate tissue (Kutscher and Wolbergs, 1935 ; Gutman *et al.*, 1936) and the caudal lobe of the rhesus prostate gland (Gutman and Gutman, 1939) contain a specific acid phosphatase in high concentration with optimum activity in the approximate *p*H range 4 to 6. Normally the enzyme appears in the prostate after puberty (Gutman and Gutman, 1938) in amounts ranging roughly from 500 to 2000 U per g. wet prostate tissue. Treatment of prepubertal rhesus monkeys with testosterone propionate causes precocious formation of the enzyme in the prostate. Prostatic acid phosphatase is secreted externally in the prostatic fluid and constitutes a chemically identifiable prostatic component of the semen. It is considered highly probable that the acid phosphatase content of the secretion of the prostate gland varies considerably in different individuals. The physiological significance of this enzyme activity of the prostate is not known, but the relation of erythrocyte phosphatase to glycolysis in shed blood suggests, state Gutman and Gutman, the possibility of an analogous relation between prostatic acid phosphatase and glycolysis in semen, and since the metabolism of human sperm is almost exclusively glycolytic, this possibility is consistent with the impression of many years' standing that prostatic secretion favours the motility of sperm.

Proteolytic agents in the semen, derived from the prostate, have been noted (Huggins and Neal, 1942).

CHAPTER 5. PHYSIOLOGY OF SPERMATOZOA

Osmotic Pressure

Gellhorn (cited by Hartman, 1939) states that guinea-pig sperm can endure a one-third reduction of the osmotic pressure of the medium, and Milovanov cites Galleoti's figures for the range of variation of osmotic pressure that sperm can tolerate, viz. : bull 1·082, dog 1·093, rabbit 0·301, and guinea-pig 0·95. Mammalian sperm, according to Milovanov, have retained the ability of adaptation to changes in osmotic pressure to a certain extent. Dog sperm, for example, die when transferred suddenly to a hypertonic (2%) solution of NaCl, but if the osmotic pressure is brought to the same level gradually, they retain their motility for a time. Slight deviations from isotonic concentrations may often not be detected by cursory examinations for activity, but their effect becomes marked in comparing survival. The use of strictly isotonic media therefore plays a great part in the practice of artificial insemination.

Roemmele considers that the determination of optimum osmotic pressure by cryoscopic measurement is not satisfactory because sperm show a considerable variation of osmotic pressure, often leading to lowered vitality of the sperm. Milovanov's results for cryoscopic estimations agree with the isotonic concentration of glucose estimated by survival of the sperm of the bull and ram, but for the stallion it is 7·2% (0·40 M) glucose by the biological method. Isotonic concentration depends on

a complex interaction between the solutes and the cell wall, *e.g.* the penetration of some substances from the surrounding medium inside the spermatozoa causes, as it were, a " release " of part of the external osmotic pressure, and the balance is only restored if the concentration of the external solution is increased. This phenomenon, Milovanov states, actually takes place in solutions of glucose and is manifested particularly in the semen of the stallion, while in the semen of other animals the results coincide with the cryoscopic data owing to the lower permeability of the membrane of the sperm of these species. No such divergence was observed with pure saline solutions, apparently due to the low permeability of spermatozoa for salts. These experiments demonstrate the importance of the biological method for determining isotonic concentrations, which enable the properties both of the cell and of the soluble substances to be taken into account.

Osmotic swelling and distension of the head have never been observed in mammalian sperm. The tails in hypertonic solutions show irregular zig-zag bends, while in hypotonic solutions, especially in distilled water, the tails are curled in rings.

Movement

Spermatozoa of farm animals and birds move, according to Milovanov (1934), by unilateral strokes of the tail in one plane, and simultaneously they rotate round their longitudinal axis owing to the asymmetrical structure of the head, thus ensuring rectilinear progressive movement. In addition to normal progressive movement there are two types of abnormal movement, rotary and asymmetrical.

The structure and motility of mammalian sperm are associated with the capacity for rheotaxis, *i.e.* orientation and movement against a flow of liquid. The development of rheotaxis is probably associated with the evolution of internal fertilisation and the orientation of sperm in the female genital tract. This capacity is associated with rectilinear movement, with which sperm alter not only their direction but also their speed when they meet a flow of liquid ; the movement is activated by a contrary flow. The sperm of the stallion, according to Yamane, are able to increase their rate of movement from 87 to 200 μ per sec. in a flow of liquid. The rate of movement varies greatly with the species, state of sperm, and the medium. The approximate rate of movement in mm. per minute is as follows : stallion 5·22 (Yamane and Ito, 1932), bull 4·02, ram 3·00, and dog 2·58 (Adolphi, 1905). Phillips (1935) found the average speed of travel of ram sperm in Ringer's and normal salt solutions was 4·6 mm. per minute. Bretschneider (1936) reported that vigorous shaking of bull semen for 3 minutes destroyed motility.

The middle piece appears to be the motor of the sperm. If the head is severed from the middle piece and tail, the latter section continues to swim in a straight line (Cody, 1925). Injury to the tail piece caused loss of motility. The proximal centriole may be the source of the power of motility (Popa and Marza, 1930).

According to Dubinčik (1934), who worked with the sperm of man, ram, stallion, and dog, motility slowed down and often ceased altogether in hypotonic solutions, while in hypertonic solutions it ceased immediately. At pH = 4·2 nearly all motility ceased but was resumed again after a shift towards alkalinity ; at pH = 3·5 to 3·4 irreversible immotility occurred. Motility increased at 35° C. in a 20% solution of gum arabic ; in a 20% gelatin solution movement was slower and ceased in a 25% solution. Weber (1936) noted that when the motility of bull sperm had ceased in storage it could be restored with isotonic salt or glucose solution. Steensma (1938) observed that when the pH of bull semen was artificially lowered before centrifuging the semen, the time of survival was increased. Herman and Swanson (1941), who investigated the interchange of sperm and seminal fluid of different bulls, found that the motility of sperm after ejaculation was more dependent on a property of the sperm themselves than upon the type of seminal fluid in which the sperm were suspended.

Resistance

The resistance (R) of sperm against the destructive action of 1% sodium chloride is an index much used by Russian workers. The value of R (*see* p. 96) varies greatly in different species and individuals (Milovanov, 1934) :—

Bull	300—20,000
Ram	100— 5,000
Stallion	100— 1,500
Boar	60— 1,000
Dog	200— 600

In experiments, mainly on ram sperm, Nagornyĭ and Smirnov (1939) found that the volume of NaCl required to kill sperm was 2 to 3 times less than that of glucose or Na_2SO_4, but greater than that of $CaCl_2$. Investigation of the effect of ion content showed that the most favourable were phosphate and tartrate (when R = 34% of its value in undiluted semen), followed in decreasing order by NaCl, $CaCl_2$ and Na_2SO_4. The authors consider that their results confirm the existence of the lipoid sperm capsule. They recommend standardisation of the technique in order to obtain comparable results.

Agglutination

Milovanov and Selivanova (1932) observed that in dilutions of over ×32, agglutination of ram sperm occurred ; the heads became stuck together and they formed star-like figures, and at the same time activity was reduced. It was demonstrated that (1) agglutination did not occur, even in dilutions of ×200, in pure isotonic glucose solutions ; its production went parallel with the replacement of part of the solution by phosphate mixtures and when the mixture consisted of 80% of phosphate buffer solution, practically all the sperm were agglutinated ; (2) agglutination was marked when sperm was diluted with isotonic solutions of alkalines, *e.g.* $NaHCO_3$, NaCl, and Na_2SO_4 ; (3) agglutination increased in a more alkaline medium ; (4) it increased with increased concentration of the solution. They consider that agglutination is of an electric nature. Non-agglutinated sperm in a glucose solution are carried towards the anode, *i.e.* are negatively charged, while agglutinated sperm in electrolytic solutions are not transported by the current. Agglutination is due to the elimination of the electric charge of the sperm by the ions of the electrolyte which have an opposite charge. Their view is that the forces of repulsion between sperm similarly charged are thus removed and the sperm agglutinate.

Effects of Temperature

Milovanov distinguished three states of a spermatozoon : motility, rest, and death. The first two are reversible and may pass one into the other ; the third may follow either the resting stage or directly the state of activity. The principal factors which determine the transition from one stage into another are the temperature, *p*H, content of electrolytes, and available energy.

Sperm are sensitive to both high and low temperatures. Sensitivity to high temperature is particularly great. In the body, temperatures higher than that of the scrotum have a harmful effect on the fully-formed spermatozoa. Outside the body, at body temperature they retain fertilising capacity for about 10 hours only, and at 45° C. they are almost instantaneously destroyed (Walton, 1930). Body temperature is most favourable for motility but not for survival. With lower temperatures the time of survival increases. This increase in survival runs parallel with a decrease in the activity of the cell, and Chang and Walton (1940) consider it probable that the increased survival is due to a reduction in the rate of those metabolic processes which lead ultimately to the exhaustion or destruction of the cell. Bernstein (1933*a*) noted that low temperature caused reduction in the rate of glycolysis.

Rate of Cooling.—Milovanov (1934) states that in glucose-phosphate media motility ceased completely at 0° C. and was most marked at 20-40° C. The fall of motility associated with a fall of temperature may be completely reversible, partially reversible, or irreversible in accordance with the rate of cooling. Rapid cooling causes more or less irreversible changes which might lead to death. This harmful effect of sudden cooling, called " temperature shock " by Milovanov, is of great practical importance and necessitates careful regulation of the temperature of artificial insemination laboratories. Birillo and Puhaljskiĭ (1936) recommended the use of a vacuum cup for the collection of bull semen. Salisbury and Willett (1940) designed an artificial vagina for the bull to prevent chilling of the sperm in cold weather and with its use obtained much better motility than with the ordinary artificial vagina.

Birillo and Puhaljskiĭ investigated the effect of cooling on bull and ram sperm. A fall in temperature from 40° to 0° C. within 30 to 60 minutes caused a rapid decrease of resistance and the loss of ability to be reactivated on being re-heated to body temperature. If cooling was continued over 2 hours, the temperature shock was less severe, while if it was prolonged over 3 to 4 hours and gradual, *i.e.* 3° C. every 15 minutes or 5° C. every 30 minutes, nearly all samples could be reactivated on re-heating. Ram sperm gave positive results only if artificially buffered. At 0° C. sperm was in a state of quiescence ; this was reached not at the moment of the fall of temperature to 0° C., but a little later. A similar lag was observed at all stages of cooling. The susceptibility of bull and ram sperm to temperature shock, the degree of which depended on the degree and rate of cooling, was also noted by Gladcinova (1937). On rapid cooling from body temperature to 5° or 10° C., activity decreased

and was not restored on re-heating; cooling to 15° C. had less effect. Sperm cooled suddenly to 5°, 10°, or 15° C. showed further reduction of activity on continued storage at these temperatures. Cooling to 20° and 30° C. did not cause reduced activity. Activity of stallion sperm was not reduced on rapid cooling to 15° C., but when cooled to 10° or 5° C. activity decreased inversely to sperm volume. Sperm which had been subjected to temperature shock perished rapidly on further storage at 38° to 39° C. These observations indicate that semen collection and insemination should be carried out at a temperature of 20° to 25° C. Cooling of sperm for transport and storage should be gradual but not too slow, since a very slow cooling may cause death of sperm through auto-intoxication.

Willett, Fuller, and Salisbury (1940) found a rate of lowering of 5° C. per 5 minutes to a storage temperature of 5° C. most satisfactory, and Davis (1938) obtained the best results by allowing an hour to lower the temperature from blood heat to 10° C. The writer, using a storage temperature of 8° to 10° C., with 15 minutes at 25° and 20° C., obtained very similar results with periods varying from $\frac{1}{2}$ to 2 hours at 15° C.

Chang and Walton (1940) studied the effects of low temperature and acclimatisation on the respiratory activity and survival of ram sperm. They found that sudden cooling had a harmful effect on the subsequent respiratory activity of the sperm, and the lower the temperature to which the semen is cooled, the greater is the harm done. It was demonstrated that the following procedures were harmful: (1) collection of semen in ordinary glass vessels during winter—sperm suffer if ejaculated into a cold receiver even at a temperature of 15° C.; (2) rapid conduction of heat from a glass bottle, as occurs when the bottle is placed in colder surroundings; and (3) adding sperm to cold diluting fluid.

In a further experiment it was found that rapid cooling (less than 5 minutes at 15°, 10°, and 5° C.) reduced the respiratory activity, measured after 24 hours' storage at 1° C., to about 20% of the fresh sample, but if kept more than 2 hours at each stage the sample lost little or none of its respiratory activity. Thus, provided the cooling was gradual, storage at 1° C. had no harmful effect. The results indicate that it is not so much the rate of fall of temperature which is the significant factor in gradual cooling as the time which the sperm remain at each given temperature and become acclimatised.

Temperature shock is more severe at a low temperature than at a higher one, and while sudden cooling is harmful at all temperatures, the effect is more marked at the lower ones, *e.g.* sudden cooling of the semen through 10° C. while the sample is otherwise undergoing gradual cooling, from (1) 25° to 15° C. gave a respiration of 70% of the fresh sample, (2) 20° to 10° C., of 60% of fresh sample, and (3) 10° to 1° C. a respiration of 50% of the fresh sample.

For short exposures temperature shock is relatively less severe than for long exposures. When semen was stored for only one hour after rapid cooling to 15° C., there was little effect on the respiration, while after cooling to a temperature as low as 1° C., respiratory activity still remained at about 45% of its original value. Temperature shock is therefore cumulative and takes some time to reach its full effect.

Rapid warming has no harmful effect upon the subsequent respiratory activity of the sperm.

It was noted that in a sample of semen subjected to temperature shock there was a decided reduction in the number of sperm which reactivated on warming and a decrease in the activity of those cells which did reactivate. No visible change in the structure of affected cells was detected.

Skatkin (1940) states that stallion sperm is very sensitive to temperature shock and that the effect is due not so much to the actual temperature as to the temperature fall, being exhibited when the fall is 13° or greater. A drop of 13° from 20° C. had a relatively greater effect than the same drop from 38° C. The degree of temperature shock varied in different ejaculates. Rapid heating (from 1° to 25° C.) had no deleterious effect on duration of survival, but a reduction was noted when sperm was re-heated to 38° C. The existence of temperature shock after heating suggests that in practice stallion sperm should be heated slowly to 20° to 25° C.

Temperature below Zero.—A fall of temperature below zero usually causes irreversible changes in the sperm. However, recent experience of various workers has shown that minute organisms and small amounts of tissue in culture media will remain alive for considerable periods at very low temperatures (*e.g.* —70° F.) provided freezing is practically instantaneous, thus preserving the colloidal nature of the protoplasm. Pursuing this suggestion, Shettles and Lamar found that sperm could be resuscitated from a bath at —70° F. after several days' exposure (Hartman, 1939). Jahnel (1938) showed that a few human sperm survived prolonged exposure to —196° C. (liquid N_2) or —269·5° C. (liquid He) and showed motility after thawing. They did not survive much shorter exposures to —10° to —20° C.

Morozov (1940) observed that bull semen may be cooled to —4° C. The critical point was

$-5{\cdot}687 \pm 0{\cdot}035°$ C. The temperature at which sperm failed to survive appeared to be closely dependent on the speed of cooling ; the quicker the cooling the higher the temperature at which death occurred and *vice versa*. Semen stored at $-1{\cdot}2°$ C. remained highly active for more than 10 days and showed signs of life for more than 3 months. Sperm remaining alive after extreme cooling had a high survival time even after repeated cooling. Keeping semen at 20° C. before cooling, with the resultant increase in lactic acid, also increased the resistance of sperm to cold, therefore preliminary storage at 20° C. for 4 hours and transport at 10° C. are recommended.

Shaffner, Henderson, and Card (1941) found that although approximately 30% of sperm in fowl semen dehydrated by the addition of laevulose and quick-frozen to −76° C. were motile when thawed at 42°-43° C. after 52 days, no fertile eggs were produced in artificial insemination tests. But semen quick-frozen at −6° C. and thawed after 30 seconds produced a live chick. The length of life of sperm preserved in the unfrozen condition was inversely proportional to the storage temperature, the most favourable being 0°-1° C. Tests below freezing showed that −76° C. was the only temperature (of those tried) at which viability persisted longer than a few hours.

Optimum Temperature

Walton (1930) found that there was an optimum temperature for storing rabbit sperm at about 12° C. ; below this some unfavourable reaction set in, which was more marked as the temperature of 0° C. was reached. An optimum temperature between 15° C. and 0° C. has been found by various workers and the lack of agreement as to the exact position on the temperature scale can probably be explained by the results of Chang and Walton (1940).

The apparent optimum temperature for the storage of sperm, they state, is affected by temperature shock and acclimatisation. The optimum depends not only on the actual temperature, but also upon the rate of cooling and the time of exposure ; with rapid cooling it is higher than with slow cooling. With very gradual cooling in stages of 5° C. and with an interval of 2 hours between the stages, the optimum temperature for ram sperm is 1° C., or possibly lower, but there was not much difference between 3° C. and 1° C. Storage at 10° C. and 7° C. respectively gave 16% and 34% of respiratory activity for 6 days. At 10° C., 7° C., and 1° C. slowly cooled semen retained 16%, 34%, and 74% respectively of its original respiratory activity for 6 days. These figures, it is stated, did not represent the full possibilities of this method of storage. Storing the whole quantity of semen in one tube gave better results than storing in small quantities. Two larger samples which were of exceptionally good quality gave 87% of respiratory activity after 13 days and 82% after 14 days.

Bull.—Bull sperm can be successfully stored at 10° C., and longer viability has been obtained with temperatures of about 2° to 4° C. (Hatziolos, 1937 ; Davis, 1938). Milovanov recommended for bull sperm a temperature of 10° C. for short periods of storage and 1° C. for longer. Davis, Underbjerg, and Williams (1940) studied the effect of storing bull sperm at temperatures of 35° F., 40° F., 50° F., and 70° F. in relation to motility, *p*H, and concentration of sperm (the rate of cooling was 1° F. per minute). The *p*H values declined during storage, the lower the temperature the slower the decline, with little difference between 35° F. and 40° F. Basing their conclusions on motility and *p*H values, they concluded that a storage temperature of 35° F. was best for bull sperm, since at that temperature the least changes occurred.

Herman and Swanson (1941) observed that the temperature of holding had a marked effect upon the rate of loss of motility in semen. Comparison of samples stored at 70° to 75° F. with those stored at 40° F. showed that at six hours semen stored at the higher temperature had slightly better activity. At 16 hours, motility in many of the samples stored at the higher temperature was very low, while in those stored at 40° F. the decline had been slight. Samples which showed excellent initial motility and retained motility very well on storage were observed to die just as fast at the higher temperature as did those of poorer quality. Hence, they concluded, the maintenance of motility in semen at this temperature was not useful in predicting motility maintenance in the same sample stored.

Frank, Smith, and Eichorn (1941) found that bull semen, undiluted or mixed with buffers, could be stored at 2° C. for 1 to 2 days, and in rare instances longer, without loss of fertilising ability. Comparing the effects of adding gelatin, buffer and nutritive solutions and of storage at low temperatures they found the most important factor was storage at 2° to 10° C.

Ram.—Milovanov (1934) stated that at body temperature glycolysis was so intense outside the organism that ram sperm died after 2 to 3 hours at an acid reaction. A reduction in temperature to 15° C. delayed acid intoxication and the period of storage could be extended to 24 hours, and at 10° C. to 2 to 3 days. Lebedeva (1934) found that undiluted ram sperm kept at 12° C. died after 36 to 48 hours through hyper-acidity ($p\text{H} = 4{\cdot}46$) ; the process was accelerated by higher tempera-

tures, while at 0° C. sperm lived for 27 days, retaining relatively high motility (0·6) for 12 to 15 days, death finally occurring through lack of energy resources. It was concluded that in practice optimal conditions for storing would be as follows: for periods up to 6 hours, keep at 10° to 12° C.; for intervals from 6 hours to several days, add 0·2 ml. phosphate buffer per 1 ml. semen and keep at 0° C.

Milovanov, Nagornyĭ, and Stoljarov (1939) found that for short periods of transport of ram sperm the comparatively high temperature of 15° to 20° C. was indicated, while for longer periods a lower temperature was desirable.

Moore, Mayer, and McKenzie (1940) observed a definite correlation between the temperature of the semen of rams and the duration of motility of the sperm. Motility ceased in 20 minutes at 45° C., in 30 minutes at 37° C., in 5 hours at 22·5° C., and in 24-48 hours between 11° and 0° C., while at 0° C. the sperm were definitely motile up to 8 days. Motility was directly affected by the degree of acidity of the semen at *p*H values below 6, ceasing altogether at *p*H 5·37, the rate of acid production in the semen being dependent in turn on temperature. It was therefore concluded that 0° C. is the most suitable temperature at which to store ram semen, since the metabolic activities resulting in the production of acid end products do not occur at this temperature, and that, if semen is to be used for insemination or storage, its *p*H should be determined as well as its motility rating.

Other Animals.—Reduction of temperature and acidification, though sufficient for inducing anabiotic conditions, cannot, according to Milovanov (1934), ensure a prolonged storage of sperm of the stallion, boar, dog, and rabbit. The life of sperm is shortened, not prolonged, at 0° C. In these species the optimum temperature for survival is about 15° to 17° C. The higher optimum temperature is said to be associated with the more rapid swelling of colloids at lower temperatures, which must also lead to a more rapid destruction of the lipoid capsule. Walton (1938*a*), however, obtained fertile inseminations with horse sperm, washed and centrifuged and stored at 0° C., but not with sperm similarly stored for 48 hours.

Milovanov, Lihačev, and Ževanova (1939) found that the optimum temperature for stallion sperm, in conjunction with other conditions, was 3° to 10° C., and that temperature did not influence survival of undiluted sperm, but on dilution it had a favourable effect, viz. at 8° to 10° C. survival was twice as long as at 20° C.; further reduction of temperature to 3° to 6° C. had no effect.

Bederke (1933) found that the optimum temperature for storage of dog sperm was 18° to 20° C., though 10° C. was not appreciably different.

Dilution

General Principles

The main object of a diluting fluid is to increase the volume of the ejaculate so that a larger number of females can be inseminated. Milovanov (1933, 1934) considered that the evaluation of diluting fluids should be based on the optimum, the maximum, and the toxic degrees of dilution. He criticised the evaluation by the estimation of survival at a random degree of dilution, as the injurious effect of some diluents is only apparent at higher dilutions. With ram sperm, for example, a large amount of salts can be introduced into the diluent when the degree of dilution is low, although such salts may have an unfavourable influence with high dilutions. It would seem that diluting fluids should preferably be tested within the range of dilution it is desired to use, allowing an adequate margin of latitude.

Diluents, however, are used not only for dilution but also for providing favourable media for the survival of sperm *in vitro*. Fluids of simple composition, but conforming to the requirements for sperm, are probably adequate for dilution *per se*, when the sperm is used immediately after collection. More elaborate fluids, which in addition attempt to supply the nutritive requirements of sperm, and which undergo as little change as possible during storage, are required for the preservation of sperm. Much can probably be learned from tissue culture work in this respect. Some such solutions may be better than simpler ones even for mere dilution. As diluting fluids are commonly tested by determining survival of sperm in them, it is convenient to discuss together fluids used for both dilution and preservation of sperm.

The following factors must be considered in the preparation of diluting fluids for mammalian sperm: (1) osmotic pressure, (2) *p*H, (3) buffering capacity, and (4) non-toxicity, electrolytes and non-electrolytes, cations and anions. In addition, diluting fluids for practical use should be as simple as possible, be easy to prepare and should keep well. *Isotonicity*: The diluting fluid must be isotonic with the sperm of a given species. *Hydrogen-ion concentration*: The *p*H must be favourable for sperm vitality. *Buffering capacity*: The buffer properties of the diluent, according to Milovanov,

are not of great importance for semen which has a low concentration of sperm, such as that of the stallion, boar, and dog. The semen of the ram, on the other hand, which is very dense and has intense glycolysis, is insufficiently buffered and on dilution with a non-buffered diluent usually perishes rapidly. Glucose-phosphate diluents are therefore used for ram sperm. The phosphate is used for its buffer qualities, despite its unfavourable influence on the capsule. Bull semen, as regards its composition and concentration of sperm, represents an intermediate condition and glucose-phosphate buffer diluents and non-buffer sulphate and tartrate diluents are equally good. Küst (1936) found that the buffering action provided by some diluents increased the motility of bull sperm. Non-buffer sulphate and tartrate diluents are considered best for the sperm of the stallion, boar, and rabbit.

Electrolytes and non-electrolytes; *cations and anions*.—The following account is taken largely from Milovanov (1934). It has been established for sperm that a certain amount of salts is necessary for normal irritability. Pure salts, however, have an injurious effect on vitality, for in such solutions sperm move very actively, but rapidly lose motility and die. It is believed that this reaction is apparently associated with the removal of the electric charge of the lipoid capsule and a change in the state of its colloids. The most favourable media are therefore those which contain a moderate amount of electrolytes, varying with the species and the electrolytes. There exists an interaction between the action of electrolytes and *p*H. Pojarkov has postulated that the optimum concentration of salts is lower, the lower the *p*H.

Referring to the influence of various ions on motility and survival of sperm, Milovanov emphasises the statement of Redenz that the ions influence not the sperm themselves but the colloids of the lipoid capsule. The ionic series obtained by various authors reproduces to a greater or less extent the lyotropic series of ions which illustrates the influence of ions upon the swelling of lyophil colloids :—

sulphate, tartrate, phosphate, citrate, acetate,
chloride, chlorate, nitrate, iodide, thiocyanate.

Anions (especially sulphate) in the first line prevent swelling and by thus reducing the destruction of the lipoid capsule delay the transition of spermatozoa into an irreversible state. Those in the second line aid swelling of colloids and by destroying the lipoid capsule cause rapid death of sperm. Dubinčik (1934) found that among the anions, citrate, then iodide and chloride were the most injurious, while sulphate and tartrate appeared to have a favourable effect. The difference in the effect of the various cations, contrary to the anions, upon the vitality of sperm is much less clear. It is a specific property of sperm that the antagonism of cations, which plays an important part in the stimulation and the normal processes of cells in general, is relatively unimportant for the physiology of mammalian sperm. This is apparently associated, Milovanov states, with the lipoid capsule which isolates the sperm from the direct influence of the medium. In media such as glucose-sulphate diluents, the antagonism of cations is not manifested. Under conditions in which the lipoid capsule is destroyed, on the other hand, as for example at high degrees of dilution with alkaline glucose-phosphate diluents, or in a medium rich in colloids, the antagonism of the ions is manifested as in other cells. According to Dubinčik the cations which exercised an injurious effect could be arranged in the following order : Fe, Zn>>>Ca, >NH_4>Na, >>Ka. A comparison of sulphate and tartrate diluents demonstrated the predominance of physical properties of a salt over the chemical, for although different in composition, their action is almost identical.

Salts such as chlorides should not be used in diluting fluids. Physiological salt solution is stated by Milovanov to have a destructive action on the capsule, and it is on this fact that the method of determining the resistance of sperm by titration with 1% NaCl is based. Both a pure solution of NaCl and one equilibriated by the addition of antagonistic cations, K, Ca, and Mg have essentially similar effects on the capsule, which is regarded as the explanation of the injurious effect of various physiological salt solutions, and also of accessory secretions. Milovanov states that Selivanova clearly demonstrated that the capsule is destroyed by the accessory secretions. This was done most easily in the ram at a dilution of ×4, slightly less so in the stallion and the boar (dilution ×16), while the sperm of the bull, which was the most resistant, required a dilution of ×256. There is an inverse relationship between the amount of accessory gland secretion in the semen and the duration of life of sperm. The semen of the bull and ram, for example, contains about 1 to 4 $\times 10^9$ sperm per ml. which survive, *in vitro*, on the average about 3 to 5 days, whereas that of the stallion, boar, and rabbit, which is greatly diluted during ejaculation by accessory gland secretion, contains only 100-500 million sperm per ml. which rarely survive more than one day. Milovanov therefore concludes that neither physiological media nor the chemical composition of seminal fluid can serve as a standard for the preparation of diluents, and the only approach is to study the characteristics of the sperm themselves.

With horse and boar semen, removal of the seminal fluid and concentration of the sperm has resulted in greater survival on storage (Walton, 1938*a* ; McKenzie, Lasley, and Phillips, 1939), but a similar procedure was not effective with bull semen (Swanson and Herman, 1941*b*).

The optimal ratio between electrolytes and non-electrolytes changes in accordance with the greater or less stability of the lipoid capsule, and the amount of the electric charge. The most resistant is the capsule of the bull and the optimum ratio of electrolytes to non-electrolytes in diluents for bull semen is given by Milovanov as 8 : 2 ; where the charge is low and agglutination occurs easily the ratio is 1 : 9. The optimum proportion of salts depends on their character, since the less they affect the capsule the more of them can be used. Glucose, which also serves as a source of energy, is commonly used as a non-electrolyte.

Milovanov investigated the effect of protective colloids on sperm. The colloids were arranged in the following ascending order of their protective effect—(1) gum, (2) gelatin, (3) egg albumen, (4) alkaline egg albumen, (5) alkaline albumen from meat or blood serum, and (6) mucin from boar semen. Mucin was the most effective and it is considered that one of the functions of mucin in the oestrous secretions of the female consists in preventing agglutination of sperm. Gunn (1936) observed that in the semen of rams mucin appeared to exert a protective influence on the sperm and favoured survival *in vitro*. The high acid-combining power of mucin, Gunn suggests, may be partly responsible for increased survival. A marked increase in the proportion of mucoid material occurred in ejaculates collected from animals after previous injection with pituitary and pregnancy urine extracts, and a less well-marked increase was noted after injection with acetylcholine and pilocarpine.

Nutrients.—Bernstein (1933*b*) found that the addition of nutrient substances to diluents for bull semen did not greatly increase the survival of sperm. Diluents using egg yolk and buffers have, however, greatly increased the survival of bull sperm (Lardy and Phillips, 1939 ; Salisbury, Fuller, and Willett, 1941).

Fluids for Dilution and Storage

Preparation of Diluting Fluids.—Chemically pure products made up in distilled water (preferably twice distilled, and boiled before use) should be used for the preparation of diluting fluids. Where there is any precipitate, as in GPS-8, it should be filtered. Diluting fluids are conveniently made up in ampoules and they can be sterilised by heat. Glucose can be incorporated when the fluid is prepared or it can be added at the time of use. Before issue of each batch of diluting fluid, it is as well to test its reaction and its effect on survival of sperm.

Sulphate and Tartrate Diluents.—

Animal.	Diluent.	Glucose, Anhydrous.	Sodium Sulphate, Anhydrous, Na_2SO_4.	Peptone Salt-Free.
Bull	SGC-2	12·0	13·6	5·0
Stallion . . .	SGH-2	57·6	3·4	2·0
Boar	SGP-2	46·1	2·8	3·5
Rabbit . . .	SGR-2	39·0	3·5	2·0
Animal.	**Diluent.**	**Glucose, Anhydrous.**	**Sodium Potassium Tartrate, $KNaC_4H_4O_6$.**	**Peptone, Salt-Free.**
Bull	TGC-2	12·0	25·6	5·0
Stallion . . .	TGH-2	57·0	6·7	2·0
Boar	TGP-2	46·1	5·6	3·5
Rabbit . . .	TGR-2	39·0	7·0	2·0

The quantities are given in grams per litre of distilled water.

These are regarded as equally good and are used for the semen of the bull, stallion, boar, and rabbit. Witte's peptone has been used successfully by the writer instead of salt-free peptone in the tartrate bull diluent. Swanson and Herman (1941*b*) found that the SGC-2 dilutor (and also 3

and 5% glucose) in dilutions of 3 parts dilutor to 1 part bull semen were definitely harmful to sperm as measured by their effect upon motility and maintenance.

Milovanov (1934) used the following fluid for producing artificial anabiosis of stallion sperm :—

Glucose	64·8 g.
Pot. tartrate	3·38 ,,
Tannin	0·02 ,,
Tartaric acid	0·10 ,,
Peptone	2·00 ,,
Water	1 litre

A new diluent, TGH-6, which increased survival, was evolved by Milovanov, Lihačev, and Ževanova (1939). It has the following composition :—

Anhydrous glucose	6·85 g.
Pot. sod. tartrate	0·15 ,,
Tartaric acid	0·008 ,,
Distilled water	100 ml.

The optimum dilution is 1 : 3.

Šergin and Gladcinova (1938) after tests with various diluents, including those containing gelatin, mannite, glycocoll or albumen, decided that the best for horse sperm was one composed of glucose, 5·8 g., and anhydrous sodium sulphate, 0·34 g., per 100 ml. distilled water. The dry components can be stored for long periods and dissolved as required. In insemination tests a dose of 20 ml. (diluted $\times 4$) gave a conception rate $2\frac{1}{2}$ times greater than with undiluted semen (dose 10 ml.) though the number of sperm was only half as great.

Freiberg (1935) recommended the following diluent for dog sperm : glucose 34 g., potassium sodium tartrate 11·3 g., salt-free peptone 3 g., distilled water 1000 ml.

Phosphate Diluents.—Milovanov regarded the phosphate dilutor as the best for ram semen.

Animal.	Diluent.	Glucose, Anhydrous.	Disodium Phosphate, $Na_2HPO_4\,12H_2O$.	Mono-Potassium Phosphate, KH_2PO_4.	Calcium Lactate, $CaC_6H_{10}O_6\,3H_2O$.
Ram	GPS-8	50·4	6·78	0·15	1·91

Winters *et al.* (1938) found that ram semen is preserved longer if an equal volume of the following solution is added :—

$Na_2HPO_4\,12H_2O$	15·4 g.
KH_2PO_4	3·2 ,,
Distilled water	1000 ml.
$CaSO_4$	Saturate

The diluent used for stallion semen by Winters and his colleagues is :—

Glucose	50·0 g.
$Na_2HPO_4\,12H_2O$	1·54 ,,
KH_2PO_4	0·32 ,,
Distilled water	1000 ml.
$CaSO_4$	Saturate

The stallion semen is centrifuged and the supernatant fluid removed. An amount of diluting fluid equal to half the original amount of semen is added to the sperm. This is again centrifuged and the fluid poured off. Diluting fluid twice the volume of the sperm cells is now added ; it is covered with oil and cooled to 41° F.

Sodium Chloride.—0·9% NaCl for bull sperm and 0·8% for ram sperm has been used successfully by McKenzie and Steensma (Lambert and McKenzie, 1940). The writer has used 0·9% NaCl successfully for sheep insemination.

Glucose.—Miller found a 3% to 5% solution of glucose the most satisfactory for bull semen (Winters *et al.*, 1938).

Egg-Yolk Phosphate and Citrate.—Egg-yolk lecithin was added to diluting fluids with beneficial results by Milovanov and Selivanova (1932). Lardy and Phillips (1939) developed a very satisfactory diluting fluid for bull sperm, made up as follows :—

KH_2PO_4	0·2 g.
Na_2HPO_4 $12H_2O$	2·0 ,,
Distilled water, boiling	100 ml.

After the mixture has cooled to room temperature, an equal volume of fresh egg yolk is added. Up to 3 parts of this egg-yolk phosphate mixture can be added to 1 part semen. The mixture should be made up freshly as required.

One serious disadvantage of the yolk-phosphate diluent is the presence of large fat globules which make it impossible to see the individual spermatozoa under the microscope (Salisbury, 1941). A new type of diluent was therefore developed, composed of equal parts of egg yolk and a M/15 solution of sodium citrate, which clears the yolk. Scherstén (1936) had previously found that the addition of sodium citrate to a Ringer-phosphate solution at the rate of 30 to 60 mg. per 100 ml. increased the longevity of sperm suspended therein. Salisbury, Fuller, and Willett (1941) added 4 or 5 parts of egg-yolk citrate diluent to one part of semen for storage.

The Missouri Agricultural Experimental Station (Lambert and McKenzie) recommend for horse and jack semen the use of the same egg-yolk phosphate buffer as for the bull, except that 10 g. glucose is added to it. One part of semen is diluted with one part of this glucose-egg-yolk buffer. Another diluent for stallion and jack semen is prepared by dissolving a ½-ounce gelatin capsule containing 5·76 g. glucose, and 0·67 g. Rochelle salt in 100 ml. water and adding 1 egg yolk (Berliner, 1942). This fluid can be pasteurised for sterilisation. It can be added to semen in a proportion of up to 1 : 10 for use shortly after collection of semen ; for storage a dilution of 1 : 3 seemed more adequate. Pregnancies were obtained with semen stored for 24 and 48 hours.

Gelatin.—Knoop (1941) obtained good results in favour of gelatin as compared with non-gelatin diluents. The gelatin diluent was composed of :—

Gelatin	2·14 g.
KH_2PO_4	0·2 ,,
Na_2HPO_4 $12H_2O$	1·325 g.
Sterilised, distilled water	100 ml.
Fresh egg yolk	100 ,,

The non-gelatin diluent was the same with the exception of the gelatin. The material other than the egg yolk was dissolved in water before adding the egg yolk. The range in *p*H was 6·7 to 6·85. The semen was diluted 4 times and stored at 4° to 6° C. The gelatin-diluted semen maintained some life for an average of 21·5 days, compared with 14½ days in the non-gelatin mixture. In a further series of experiments the diluted semen was gradually cooled from 36° C. to 5° C. 73% of sperm were motile after 2-4 days in the gelatin diluent, compared with 59% in the non-gelatin mixture, and 50% motility was observed for 12·5 days in the former and for 8·3 days in the latter. A few sperm were kept alive for 25 to 38 days. Previously the life of bovine sperm in a diluent was about 13 days (Komarov and Gladcinova, 1937 ; Phillips and Lardy, 1940).

Milovanov (1938) has investigated the use of gelatinised diluents. They consisted of glucose-phosphate or glucose-sulphate diluents, in which a certain amount of gelatin has been dissolved, giving upon setting a jelly-like consistency. The glucose-phosphate diluents are as follows :—

	GPS-2-G. Sheep.	GPC-3-G. Cattle.
	g.	g.
Na_2HPO_4	2·08	1·70
KH_2PO_4	0·08	0·07
Na_2SO_4, anhyd.	...	0·08
Glucose, anhyd.	3·20	2·85
Gelatin	5·00	5·00
Distilled water	100 ml.	100 ml.

The sulphate diluent for bull semen (SGC-1-G) is composed of :—

Na_2SO_4, anhyd.	1·36 g.
Glucose, anhyd.	2·20 ,,
Gelatin	5·00 ,,
Distilled water	100 ml.

The sulphate diluent must be neutralised with a deci-normal solution of NaOH till it reaches a *p*H of 7·5, since the gelatin gives it an acid reaction unfavourable to the sperm. The gelatin content in bull semen diluents can be increased to 10 to 20%.

A diluent GPS-7-G which has a greater content of buffer phosphate as against a smaller quantity of glucose (ratio 9 : 1 as against 5 : 5 in the GPS-2 diluent) was tried with less satisfactory results. GPS-7-G, being too alkaline and rich in salts, exerted an unfavourable influence on the viability of the sperm.

The quality of the gelatin used was of particular importance. Iso-electric gelatin, prepared by precipitating a solution of gelatin with alcohol at a *p*H of 4·8 and then washing and drying in a dessicator, gave better results than gelatin from other sources.

Milovanov recommended the following method of preparation : place the quantities of glucose, salt, and gelatin required for 100 ml. of water in a glass flask of 200 ml. capacity, add 100 ml. distilled water and heat in a water bath to between 50° C. and 60° C. until the reagents are dissolved. Soak a filter paper (ash-free) in a filter funnel 12 to 15 cm. in diameter with 50 ml. of boiling distilled water and as soon as the water has flowed away, filter the diluent; the filtration should not last more than 10 minutes.

An inverse relationship between the temperature of the diluent when added to the sperm and fertility was noted. This was attributed to variation in resistance. A too hot diluent reduced the resistance of the sperm. The temperature of the diluent should not therefore exceed 28° to 29° C. in practice. The lower limit of the optimal zone was not clear, but it must not be below 22° C., at which temperature solidification of the gelatin begins.

A new diluent must be prepared every day, as it cannot be preserved for longer than one working day.

Blood Serum.—Bull sperm is agglutinated by blood serum of the cow and bull (Bernstein and Lazarev, 1933). The process, however, was reversible and the sperm regained motility in about 24 hours. Serum inactivated by heat had a favourable effect on the retention of activity.

Tissue Extract.—A significant advance in prolonging the viability of bull sperm *in vitro* has been obtained by Frank, Smith, and Eichorn (1941). Tissue culture work has shown the importance of embryonic tissue extract for promoting cell growth and multiplication (Parker, 1939). Bull sperm preserved at 2° C. in embryonic tissue extract prepared from developing chick embryo contained motile sperm for periods up to 46 days ; in one experiment live sperm were observed up to 22 days in tissue extract compared with 6 days in egg-yolk buffer solution. At 37·5° C. sperm suspended in embryonic tissue extract retained approximately the initial motility for 6 hours, whereas in the yolk-buffer suspension they were dead at the end of 3 hours.

Other Substances.—Gunn (1936) tested the effects of various fluids on the longevity of ram sperm, as determined by the duration of motility. The substances tested were—neutral glucose saline, sterile blood serum of horse and sheep, oestrous secretion of the ewe, alkaline normal saline containing 0·89% NaCl and 0·10% $NaHCO_3$, acetylcholine, and thin sterile starch. It was only in the last two that there was any marked increase in longevity, and in the starch the motility of the sperm was somewhat reduced.

With cock semen diluted ×10 and ×20 with Tyrode solution, motility (even progressive motion) was retained for a considerable time (in some cases 120 hours) while in dilutions of 1 : 40 to 1 : 320 no progressive motion was observed and oscillatory motion ceased rapidly (Grodziński and Marchlewski, 1935). The most favourable diluent was blood serum followed (in this order) by centrifuged extract of crushed whole chick embryos, egg albumen, Tyrode fluid, and blood plasma mixed with semen coagulate. The optimum *p*H lay between 7·2 and 8·0. Motility was affected by temperature. Progressive motion decreased rapidly at 37° C. ; semen diluted with albumen retained its motility longest at 16° C., while in the other media the optimum temperature was 2° C. Munro (1938) also found that the motility of cock sperm *in vitro* depended on an interplay between temperature and the medium. Most synthetic diluents supported motility at room temperature, but inhibited it at body temperature, but movement was regained when the temperature was lowered. On the other hand, rat and guinea-pig sperm were immediately immobilised by low temperatures and were most active at body temperatures in the same media. Seminal fluid, blood serum, thin egg white, and fluid from the shell gland supported the motility of fowl sperm from 75° to 105° F., whereas fluid from the magnum

and the infundibulum behaved as synthetic diluents, immobilising sperm at 105° F. but supporting motility at lower temperatures.

Dunn and his colleagues (1942) investigated the effect of Amphyl on bull semen. This is a concentrated disinfectant consisting of a mixture of *p*-chloro-*symm*-*m*-dimethyl hydroxybenzene, *p*-*tert*.-amyl hydroxybenzene and neutral soap dissolved in alcohol, glycerol and water ; it has a phenol coefficient of 10, but is practically non-corrosive to animal tissue. When 0·2 to 0·5% Amphyl was added to semen diluted with the egg-yolk-phosphate buffer solution, sperm lived from 4 to 9 days longer than sperm in the same diluted semen but containing no Amphyl. The rate of destruction of bacteria in the control semen was about the same as that in the Amphyl-treated semen.

Steensma (1938) observed that a strychnine solution mixed 1 : 1 with immotile bull and ram semen caused motility to be resumed for 24 to 36 hours. Bretschneider (1936) noted that tutocaine reactivated sperm which had been removed from the testes or epididymes of the bull, but not those in a normal ejaculate.

Mammalian and bird sperm are highly resistant to alcohol (Bernstein and Petropavlovskiĭ, 1937). In low concentrations of alcohol, vitality was maintained even longer than in seminal fluid or salt solution.

Use of Diluents

The degree of dilution depends, among other things, on the number of sperm required for conception in a particular species. Milovanov (1934) gives the following particulars :—

Animal.	Degree of Dilution.
Cattle . . .	4-8 times, *i.e.* 1 part semen to 3-7 parts diluent
Sheep . . .	2-4 ,, ,, 1-3 ,, ,,
Horse . . .	4-8 ,, ,, 3-7 ,, ,,
Pig	4-8 ,, ,, 3-7 ,, ,,
Rabbit . . .	16-32 ,, ,, 15-31 ,, ,,

It is most important to avoid temperature shock when adding diluent to semen. The temperature of the diluent should not differ greatly from that of the semen (as a rule a temperature of from 15° to 25° C. is harmless) and the fluid should be added slowly.

Quality and Dosage of Gelatinised Sperm.—The following method was used by Milovanov (1938). The activity of the sperm is estimated immediately after collection and if it is satisfactory, *i.e.* has an activity of 5/5 or 4/4, the concentration is determined with a haemocytometer. Sperm is diluted directly in the sperm-receiver and dilution is based on capsules containing for the ram 1 ml. of diluted semen with about 500 million sperm. The following table is given for calculating the degree of dilution :—

Concentration. (thousand millions.)	Degree of Dilution.	Concentration. (thousand millions.)	Degree of Dilution.
Cattle.		Sheep.	
0·8	1 : 2	2·0	1 : 3
0·9	1 : 3	2·5	1 : 4
1·0	1 : 3	3·0	1 : 5
1·1	1 : 3	3·5	1 : 6
1·2	1 : 4	4·0	1 : 7
1·3	1 : 4	4·5	1 : 8
1·4	1 : 5	5·0	1 : 9
1·5	1 : 5	5·5	1 : 10
1·6	1 : 5	6·0	1 : 11
1·7	1 : 6	6·5	1 : 12
1·8	1 : 6	7·0	1 : 13
1·9	1 : 7	7·5	1 : 14
2·0	1 : 7	8·0	1 : 15

More concentrated semen will be diluted to a still higher degree. Different degrees of dilution of ram semen up to 13 times with gelatinised GPS-2 gave very similar fertilisation results (the mean number of sperm per dose was from 4·4 to $5{\cdot}1 \times 10^8$ and the average resistance was from 4080 to 4640).

The quality of sperm is of extreme importance. In practice, sperm to be used for transport and insemination should have an activity of not less than 0·8 (or 4/5) and a resistance of not less than 5000. The number of ram sperm for vaginal insemination is calculated in relation to the resistance; the dosage of gelatinised ram sperm is worked out as follows: the concentration of sperm in the ejaculate is determined; the ejaculate is diluted to a density of 500 million sperm per ml.; the resistance is then determined and the appropriate dose is chosen. The dose will vary from 2·4 ml. when R = 5000 to 0·4 ml. with a resistance of 30,000.

Since a method of qualitative dosage has not been worked out for the sperm of the stallion, bull, and boar, a standard quantitative dosage has to be used.

	Density. (millions per ml.)	No. of Sperm per Dose. (millions.)	Dose of Gelatinised Sperm. (ml.)
Bull (capsules)	250	1,000	4
Bull (gelatin capsules) . . .	250-500	250	0·5-1·0
Stallion	100	4,000	40
Boar	100	10,000	100

In Milovanov's opinion appropriate dosage of sperm in accordance with its quality is the essential condition of increasing fertility in artificial insemination with gelatinised sperm.

Metabolism

The metabolism of spermatozoa has been studied by measuring the gaseous exchange by manometric methods, and also by biochemical methods whereby the concentration of various substances is measured and their catabolism traced to a measurable end-product.

Respiration

Sperm can retain their activity in a medium without oxygen for a considerable time. Addition of cyanide salts does not arrest this movement. Movement of sperm in a non-oxygenated medium is not accompanied by the production of CO_2, which shows that the energy is obtained not through respiration but by some other process (Ivanov, 1931). An increase in the rate of oxygen consumption of dog sperm after the addition of prostatic fluid was noted by Ivanov (1929, 1930). The initial stimulus was followed by a rapid decrease. The heat-labile substance in the prostatic fluid which initiated the increased respiration had an opposite effect on the respiration of ground muscle.

Roemmele observed that if the medium containing sperm is covered up, as with a cover glass, activity is slower than when exposed to the air, this being due to CO_2 retention rather than lack of oxygen. Shettles (1940) found that a CO_2 concentration of approximately 20% in an atmosphere of oxygen was required to immobilise sperm in a hanging drop. Revival of activity depended on the length of time the sperm were immobilised, but all concentrations causing immotility were toxic provided the sperm were exposed sufficiently long. The CO_2 content of the tissues of the rat was much lower than that necessary to immobilise sperm *in vitro*; the concentration in the tissues of the genital tract ranked in the following decreasing order—vas deferens, epididymis, testis.

Sperm motility certainly does not depend on the presence of oxygen; Milovanov (1934) considers that this independence undoubtedly plays a very important part under conditions of internal insemination when the spermatozoon must travel a long distance along the female genital tract, which is poor in oxygen.

MacLeod (1939, 1941*a*) found that oxygen consumption of human sperm was very small. It was shown that cyanide (10^{-3} M) does not inhibit oxygen consumption when the latter is detectable. Also motility was not depressed in the presence of cyanide or carbon monoxide. Consideration of these facts, together with the maintenance of motility under anaerobic conditions, indicates that the motile activity of human sperm is not dependent on energy derived from respiration.

All actively respiring tissues, when examined spectroscopically, under anaerobic conditions, show the absorption spectrum of reduced cytochrome (Keilin, 1925). Spectroscopic examination of a concentrated suspension of human sperm showed that if cytochrome is present in these cells it does not exist to any marked degree and no bands of reduced cytochrome were seen. MacLeod remarked that it is not at all certain that the small oxygen consumption of human sperm is operating through a cytochrome system, or indeed that these cells possess, to any degree, any of the haemin systems now known to be essential components of the main respiratory pathways in living cells. However, MacLeod (1943*b*) later demonstrated that human sperm contain the complete cytochrome complex and also an active succinic dehydrogenase. Respiratory inhibitors such as cyanide, azide, and carbon monoxide do not depress motility (MacLeod, 1941*a*), but the glycolysis inhibitors monoiodoacetate and fluoride inhibit glycolysis and destroy motility (MacLeod, 1941*b*).

An inverse relationship between the age of a specimen of human semen and its oxygen uptake has been reported by Shettles (1939, 1940). The change in respiratory rate was paralleled by a decrease in the respiratory quotient which at one-half hour was 0·93, while at 12 hours it was 0·72. Rate of respiration in semen from different donors varied directly with sperm concentration. MacLeod (1939, 1940, 1941*a*) on the other hand was unable to demonstrate an appreciable consistent uptake of oxygen, which was confirmed by Ross, Miller, and Kurzrok (1941), who indicated that this finding was possibly due to the nature of the medium in which the measurements were made. MacLeod considered that Shettles' experiments were open to question on technical counts.

Spermatozoa reduce methylene blue, and Klein and Saroka (1941) have stated that viable human sperm decolourise it. Lardy and Phillips (1941*b*) found that with bull sperm the methylene blue reduction time was greatly prolonged in the presence of glucose.

A definite relationship between the number of services per conception and the respiratory rate of bull sperm has been noted by Walton and Edwards (1938), the fewer the services per conception the greater the average initial respiration rate. Ely (1942) studied the oxygen consumption of dairy bull sperm and its relation to other characteristics of the ejaculate. Samples maintaining a motility rating of 2 for an average of 156 hours on storage at 40° F. had over twice the oxygen consumption of samples surviving less than 24 hours. Samples intermediate in survival time were also intermediate in volume of oxygen consumed. Three lots of samples grouped according to their percentage of abnormal spermatozoa had a decreasing oxygen consumption with increasing number of abnormal spermatozoa. Henle and Zittle (1941) found that preparations of crude or purified gramicidin will first stimulate and then inhibit completely the oxygen consumption of bull spermatozoa in acid-phosphate-Ringer solution whereupon motility ceases. In alkaline-phosphate-Ringer only the increase in oxygen uptake was noted. In bicarbonate-Ringer inhibition of oxygen uptake occurred, aerobic and anaerobic glycolysis being inhibited. Tyrocodine caused only small reductions in the oxygen uptake and glycolysis.

Winchester and McKenzie (1941*a*) noted that the seminal fluid of boars showed a respiratory activity of 5-22% of that of whole semen with an R.Q. of unity. The metabolic rate of the ejaculate of a bilateral cryptorchid was of the same order as that of normal seminal plasma.

Windstosser (1935) made observations on sperm from the epididymides of the bull, guinea-pig, and rat. In undiluted sperm the rate of respiration ranged from 0 to 3·815 ml. per 10 million sperm ; the mean curve of absorption was parabolic, the highest point, 1·668 ml., being reached at the end of the second hour ; during the third hour respiration was negligible. In sperm diluted 1 : 200 with saline solution the range was 0 to 10·06 ml. ; the curve rose most during the first hour, but subsequently continued to rise, the maximum of 5·283 ml. being reached at the end of the third hour. In both series the sperm were immotile throughout the experiment. Lardy and Phillips (1941*a*) found that addition of glucose reduced the oxygen uptake of bull sperm, and Henle and Zittle (1942) that glucose inhibits the respiration of bull semen but enhances the respiration of sperm from the epididymis, which indicates a change in the metabolic requirements of seminal sperm compared with epididymal sperm.

No definite correlation was noted by Windstosser between respiratory rate and motility or *p*H of epididymal sperm. Comstock (1939) observed a correlation between the duration of motility and respiratory activity of ram sperm in normal semen. Winchester and McKenzie (1941*c*) found that respiration rates of ram and boar sperm were definitely influenced by the *p*H, the optima being 7·0 to 7·2 and 7·2 to 7·3 respectively, and (1941*b*) that at low cell concentrations a decrease in the oxygen consumption per unit quantity of sperm invariably accompanied an increase in the number of sperm per ml. Windstosser also noted that greater density caused lower rate of respiration and thus lower CO_2 output, which was regarded as contradicting the hypothesis that the closely packed masses of sperm in the epididymis elaborate large amounts of CO_2 and so raise the *p*H of epididymal

secretion. Ivanov (1936) found an approximate R.Q. in ram semen of 1·0 in the presence of glucose and about 0·78 in its absence. Šergin (1939) found that the oxygen consumption of the sperm of various farm animals varied with change in temperature, *p*H and sperm concentration. When ram sperm was diluted to give approximately the same concentration as bull, boar and stallion semen, the oxygen consumption was the same for all four species. He found that 29 ml. of oxygen were consumed by one gram of sperm in one-half hour at 38° C., compared with 50 to 270 for muscle, 150 to 160 for liver, and 110 for brain. Lardy and Phillips (1941*b*) noted that the respiration of washed bull sperm falls off rapidly after the first half hour of incubation at 37° C. This decrease in oxygen consumption coincided with a decreased number of motile sperm.

Chang and Walton (1940) studied the effects of low temperature on the respiratory activity of ram sperm. The total oxygen consumed in two hours by a fresh sample at 37° C. varied from 0·247 to 0·327 ml. and per million sperm from about 0·47 to 0·69 mm^3. There was close agreement between the motility of the sample and the oxygen comsumption, *e.g.* a sample of high respiratory activity showed nearly all the sperm in rapid progressive motion, and a sample which had only 20% of its original oxygen consumption showed a large proportion of the sperm dead and only a small number feebly active.

Glycolysis

An essential condition for the retention of motility of mammalian sperm in a non-oxygenated medium is the presence of sugar ; in its absence motility ceases, but is resumed whenever sugar is added to the medium. According to Milovanov, Redenz, who was the first to study in detail the metabolism of mammalian sperm, describes the process as follows. The spermatozoa of warm-blooded animals show marked glycolysis, this process being apparently associated with motility. In moving spermatozoa glycolysis is very intensive, both under aerobic and anaerobic conditions, while in oxygen anaerobic glycolysis does not disappear but is merely reduced.

Glycolysis, regarded as the principal source of energy of spermatozoa, is affected by the following conditions (Redenz) : (1) glycolysis is most intense at $p\text{H} = 7{\cdot}60$, when the medium is more alkaline or is acidified to *p*H 6·4 glycolysis proceeds more slowly ; (2) a certain degree of influence of glucose on the intensity and duration of motility of sperm, which Redenz explained by the increase of glycolysis with increase of glucose concentration—in his experiments glycolysis reached its maximum at 0·25% glucose but a similar influence was produced by fructose, mannose, and maltose, while sucrose and galactose did not lead to an increase of glycolysis ; (3) a specific action exercised by the bicarbonate ion HCO_3 which in small concentrations, up to 45×10^{-3} mol, caused a rise of glycolysis.

A factor of practical importance, the nutrition of spermatozoa, *i.e.* the assimilation of energy from outside, arises from the work of Redenz which indicates that spermatozoa can move by assimilating the glucose which is introduced into the medium.

A decrease in the sugar concentration and an increase in lactic acid in bacteria-free human semen incubated at 38° C. was demonstrated by McCarthy and co-workers (1926, 1928). There was an increase in the *p*H during the first 3 to 6 hours, followed by a decrease. Ten to 25% of the original sugar remained at the end of 24 hours. In some cases the amount of lactic acid formed was not enough to account for the sugar used and they concluded that lactic acid is not the end product of glycolysis in human semen. No investigations were made of the amount of sugar lost by oxidation to CO_2 and H_2O. Very similar results were reported by Killian (1933) and also by Goldblatt (1935), who showed that the changes were dependent on the presence of active sperm.

MacLeod (1939, 1941*a*) studied the metabolism of human sperm using Warburg's manometric technique. In a few cases, chemical determinations of lactic acid by the method of Friedemann, Cotonio, and Shaffer (1927) were made, and were found to agree with the manometric readings within ±8%. MacLeod found that the metabolism of human sperm was almost exclusively glycolytic. Aerobic lactic acid production as measured by CO_2 displaced from bicarbonate (8·0 $mm.^3$ $CO_2/10^8$ cells/hour) was 80% of the anaerobic and fell off with time, whereas anaerobic glycolysis was linear over a period of 7 hours. Maximum motility was maintained for many hours under anaerobic conditions but showed a marked tendency to decrease in air or impure oxygen. This suggests that oxygen has an inhibiting effect on the normal function of human sperm.

MacLeod (1941*a*) gives figures for aerobic and anaerobic lactic acid production. The results are expressed as $mm.^3$ $CO_2/10^8$ cells/hour and as Q_g, or the amount of CO_2 per mg. of dry tissue per hour. The data for 83 individual specimens from 30 men are : anaerobic glycolysis—$mm.^3$ $CO_2/10^8$ cells/hour, 10·6 (27·2-3·5) and mean $Q_g^{N_2}$ 7·4 ; aerobic glycolysis $mm.^3$ $CO_2/10^8$ cells/hour, 8·5 (22·8-3·2) and mean $Q_g^{O_2}$ 5·9 (22·8-3·2). The acid production of any one individual remained relatively constant

from week to week. Sharp rises and falls occurred from time to time which could not be accounted for on the basis of motility, since all the sperm showed maximal and sustained motility. Whether the glycolysis was high or low, the ratio between aerobic and anaerobic glycolysis remained relatively constant, though in some cases there was no evident difference between aerobic and anaerobic metabolism.

There were striking differences in lactic acid production between individuals, which could not be accounted for on the basis of motility. The acid production of one individual, for example, was about 4 times that of another and the glycolysis of one did not tend to approach that of the other even when one was high and the other low. No significant difference was found in relation to cell morphology and at present there is no evidence to explain the quantitative metabolic differences between specimens of different individuals and those which may be found from time to time in the same individual.

In a typical experiment, over a period of 7 hours, while anaerobic glycolysis was linear over the entire period, aerobic glycolysis tended to fall off slightly with time, this decrease becoming apparent only after several hours in most specimens and coinciding with a fall in motility. Under certain experimental conditions, human sperm are sensitive to injury in the presence of oxygen, as the evidence of failure of motility and of lactic acid production shows. In this typical experiment at the end of 7 hours under identical conditions, motility in nitrogen was maximal whereas in oxygen it was zero.

Added substrate in the form of utilisable sugar is essential for the maintenance of glycolysis and motility of human sperm. Maltose, mannose, fructose, and glycogen can be substituted for glucose with no evident difference in the level of glycolysis or motility, but sperm cannot utilise lactose, sucrose, or galactose and when these sugars are substituted for glucose, glycolysis and motility fall rapidly. MacLeod found the effective concentration of utilisable sugar to lie between 20 and 200 mg.%; at higher sugar levels the sperm showed signs of toxicity indicated by failure of motility.

The presence of bacteria in sperm suspensions at 38° C. is usually not manifested until between the 3rd and 6th hours. Bacterial growth shows itself by an increase in lactic acid production which proceeds logarithmically. The metabolic figures affected are those of anaerobic glycolysis. MacLeod demonstrated that the metabolic figures of the human sperm in his work were not affected by bacteria during the early hours of the experiments.

Bernstein (1933*a*, *b*) found that when bull semen was stored at 12° to 14° C., the glucose content gradually decreased, the rate of decrease being irregular, greatest in the earlier stages and slowing down subsequently, *e.g.* the sugar content fell to 78% in the first 6 hours ; at the 24th hour to 47% and at the 72nd hour to 36% of the original amount. After $2\frac{1}{2}$ days of storage only 2% of the original amount of glucose was split in 6 hours compared with 22% in the same period at the beginning of the experiment.

When the activity of the spermatozoa is highest the curve of glucose splitting is steepest, while during the last hours of survival, when only single spermatozoa showing oscillatory motion can be seen, the curve begins to approach the horizontal. Motility ceases when there is still a considerable amount of glucose present. There is no obvious correlation between activity and length of survival of spermatozoa and glucose concentration. The reduction in motility takes place at different levels of sugar concentration. Bernstein noted that glucose is split in semen which contains only immotile spermatozoa, so he concludes that, apparently, the activity and motility of spermatozoa are not essential factors for glucose splitting, although they influence the intensity of this process.

Bernstein's experiments clearly demonstrated that glycolysis occurs in bull semen and that this is largely due to the presence of spermatozoa. In one experiment, for example, the amount of glucose in whole semen was reduced by 57% during 24 hours, while in the semen plasma only a little over 10% of sugar was split during this period. The splitting of glucose may be the result of fermenting properties of the semen itself or of the activity of micro-organisms, but Bernstein states that micro-organisms apparently do not greatly influence the splitting of glucose by the sperm.

Bernstein showed that spermatozoa, like muscle, possess the ability of splitting glucose in the absence of oxygen. Under conditions of oxygen starvation spermatozoa retained an approximately normal ability of splitting glucose for a fairly long time, and only at later stages of survival (after 24 hours) did the difference gradually increase between the curves of splitting sugar in normal semen and in semen (1) in which the air in the semen tubes was replaced by hydrogen and (2) treated with cyanide or ether. For example, after 24 hours at 12° C. the glucose in normal semen was reduced to 77% of the original amount, while in (1) and (2) it was only reduced to 85% and 94% respectively. During the earlier hours of survival the spermatozoa exhibited active motility, while later a reduction in activity set in, earlier than in normal semen. Ether reduced the activity of spermatozoa and the

splitting of glucose. It had a more marked effect on the intensity of carbohydrate metabolism than had cyanide. The rate of glycolysis was increased at higher temperatures, *e.g.* after 24 hours' storage at 12° C. glucose was reduced to 77% of the original amount, while at 36° C. it was reduced to 47%. According to Šergin (1937) bull semen still contained over 60% of its sugar after 24 hours. The increases in lactic acid corresponded to the sugar losses, but were not sufficient to account for all of this substance metabolised. The same author found that ram sperm utilised almost all the available sugar in 6 hours. Comstock (1939) found that the rate of glycolysis per 10^9 ram sperm ranged from 0 to 1·8 mg. per hour. Significant differences independent of percentage of motility and cell number were established between the glycolytic powers of fresh semen from different males. A high positive correlation ($r = 0{\cdot}88$) was found between glycolysis of fresh sperm and persistence of motility during storage. Glycolysis decreased as the sperm aged. The author regarded the measurement of glycolysis as the best criterion yet discovered for predicting the potential motility of fresh sperm.

The figures given by Lardy and Phillips (1941*b*) for aerobic and anaerobic glycolysis are, states MacLeod (1943*a*), virtually identical and quantitatively similar to the figures for human cells. Henle and Zittle's (1942) figures for the same cells, however, are very much higher and comparable to those of Redenz (1933). The latter workers find the aerobic glycolysis to be only about 55 per cent. of the anaerobic.

Moore and Mayer (1941) found a definite influence of temperature upon the rate of glycolysis in ram semen. Glycolysis did not exist at 0° C., but began at temperatures just above zero ; the rate increased rapidly with temperatures up to 45° C. The loss of motility and of sugar frequently coincided but the activity of spermatozoa was not entirely dependent on the sugar for (1) activity might continue for some time after the sugar had disappeared, and (2) motility was seen to cease in the presence of about 150 mg. sugar per 100 ml. semen. Moore and Mayer concluded that no direct relationship between motility and sugar concentration exists and that a factor or factors different from, but possibly related to, the sugar concentration may be responsible for the changes in the rate of motility.

The factor which influences motility and glycolysis rates is the degree of acidity or the hydrogen-ion concentration of the medium surrounding the sperm cells. The increase in *p*H is the direct result of the accumulation in the semen of metabolic products, of which lactic acid is the chief if not the only constituent.

Bernstein and Slovohotov (1933) studied the lactic acid metabolism of bull and human sperm, using the method of Friedemann, Cotonio and Shaffer, supplemented by the colorimetric method of Mendel-Goldscheiner. The lactic acid content of fresh sperm was fairly high, varying from 40 to 50 mg.%. The values for different bulls and for different ejaculates from the same bull were variable, the variation between extremes being 100% or more ; this variation may be associated with density of sperm, interval between matings, and the time between collection of ejaculates and examination. When semen was stored there was a considerable increase in lactic acid, *e.g.* from 45 to 81 mg.% in 24 hours. Together with this there was a gradual reduction in motility, ending in complete immotility, but not necessarily correlated with a definite concentration of lactic acid, since in some cases motility ceased at a concentration of 73 mg.%, while in others it was retained at 85 mg.%.

The curve of glycolysis during the first few hours of survival outside the body rises rapidly owing to the intensive formation of lactic acid, but subsequently the intensity decreases and in some cases there is even a slight reduction in the total quantity of lactic acid.

In semen from which all sperm were removed by centrifuging, glycolysis also occurred but very slightly, *e.g.* lactic acid increased from 45 to 58 mg.% during 20 hours, whereas in whole semen it rose to 81 mg.% in the same period. Micro-organisms may have been partly involved in this change.

The intensity of glycolysis in semen depends on temperature, low temperature having a depressing and high temperature an intensifying effect, *e.g.* after 6 hours at 10° C. the percentage increase in lactic acid was 118 and the motility 100%, while after 6 hours at 37° C. the percentage increase in lactic acid was 203 and the sperm were immotile. The immobilisation of glucose by heat (15 minutes at 55° C.) is accompanied by a disturbance in the formation of lactic acid.

Under conditions of anoxybiosis (poisoning with NaCN) the amount of lactic acid increased greatly, *e.g.* in 21 hours by 180%, while during the same period the increase in normal semen was only 80%. With NaCN asphyxiation there was a reduction in motility and a shortening of the period of survival of sperm, *e.g.* after 9 hours only 20% of sperm retained a slight oscillatory motion and after 21 hours immotility was complete. Bernstein and Slovohotov remark that since at low temperatures motility and metabolism of sperm are relatively slow, no marked difference is observed between the rate of splitting of sugars and respiration ; when the sperm are poisoned by NaCN, the

partial blockage of the oxidation renders elimination of the products of splitting more difficult and when sperm are stored at body temperature the rate of activity and metabolism apparently increase incommensurately with the " capacity " of the oxidation system.

Šergin (1935) found that boar and stallion semen taken from ducts before ejaculation contained small amounts of lactic acid. This was attributed partly to a slow process of glycolysis during anoxybiosis and partly to the activity of the duct tissues.

Bernstein and Šergin (1933) were led to the study of lactacidogen, a labile ester of hexose and phosphoric acid, from the work of Bernstein, and Bernstein and Slovohotov, which indicated that the processes of splitting and utilisation of carbohydrate are of the type of Meyerhof's reaction, in which the immediate link is the so-called lactacidogen. They investigated the semen of the bull, stallion, dog and man, using a modification of the method of Embden and Jost. The lactacidogen in mg. per 100 ml. semen was : bull 104·2 (11 determinations), stallion and dog 81·9 and 8·1 mg. respectively (1 determination each). In 10 different bulls the amount in fresh semen varied from 64 to 136 mg.%. The variation during storage was not parallel to that of glucose and lactic acid ; the concentration of lactacidogen exhibited irregular rises and falls which might attain 30 to 80% of the initial quantity. Cessation of motility was not accompanied by a detectable reduction in the amount of lactacidogen, so that immobilisation of sperm could not be ascribed to exhaustion of the store of lactacidogen. Heating of sperm to 55° C. for one-half hour completely arrested splitting of lactacidogen and therefore the carbohydrate metabolism of sperm.

The chemistry of the function of sperm is considered by Redenz to resemble closely muscle chemistry, the principle difference being the preponderance of the splitting up process over the oxidising, which renders the sperm incapable of eliminating completely lactic acid formed during glycolysis. The chemical changes accompanying muscular contraction (Wright, 1941) are exceedingly complex and cannot be discussed here in any detail. The outstanding changes are a decrease in the amount of creatine-phosphate (phosphagen) and an increase in creatine, inorganic P and lactate. Under anaerobic conditions lactic acid is formed quantitatively from glycogen. The intermediate stages, which are very complex, involve the temporary formation, first of hexose phosphates and then of triose phosphates, and ultimately both lactic acid and phosphoric acid are liberated ; lactic acid accumulates as lactate. In aerobic conditions the lactate is rapidly disposed of, but only when the muscle is alive. Therefore the removal of lactic acid is an oxidative process and is dependent on the intervention of living tissue.

Torres (1935) compared the ability of extracts of a variety of tissues to bring about the reaction between phosphopyruvic acid and creatine to form phosphocreatine and pyruvic acid in the presence of adenylpyrophosphate. Next to muscle, the testis gave the most active extracts. The activity of the testes is associated with the sperm, and Torres suggests the possibility that phosphocreatine plays a similar rôle in the motility of the spermatozoon and in the contraction of muscle.

Lardy and Phillips (1941*c*) found that egg lecithin decreased the respiration and greatly prolonged the motility of bull sperm suspended in Ringer phosphate medium. The decrease in oxygen consumption coincided with the decrease in motility. Phospholipids from rat liver, lecithin from soybean and cephalin from soybean or egg yolk were utilised by bull sperm. Oxygen consumption was not appreciably increased by lecithin additions to a medium containing glucose ; this, state Lardy and Phillips (1941*d*), is in agreement with previous conclusions that the intracellular reserves of the sperm are phospholipid in character.

Ivanov (1931, 1935) had found (1) that sperm remained motile in a medium containing sufficient cyanide to inhibit respiration almost completely, and (2) that motility was retained in a Ringer's solution containing sufficient monohaloacetic acid (0·002 to 0·004 M) to inhibit lactic acid formation from glucose. He therefore concluded that sperm derives its energy from some process other than oxidation and that motility does not depend on glycolysis. Lardy and Phillips (1941*b*) confirmed these observations for bull sperm and in addition showed that sperm retained their motility for some time when glycolytic and oxidative mechanisms were blocked simultaneously. It is evident, they state, that a process other than oxidation or glycolysis can furnish energy for motility.

The study of the effects of inhibitors on sperm demonstrated that (1) low concentrations of iodoacetic acid completely inhibited the breakdown of glucose to lactic acid, but did not directly affect motility ; (2) selenate, arsenate and malonate, at concentrations of 0·01 M, did not affect motility ; malonate inhibits succinic dehydrogenase, the most active dehydrogenase in sperm ; it would therefore seem that succinic dehydrogenase is not concerned with the maintenance of motility, but it may function in the oxidative removal of waste products ; the same levels of selenite, arsenite, fluoride and H_2O_2 proved to be highly toxic ; (3) 0·001 M cyanide inhibited the motility of sperm in

yolk-buffer, but had little effect on motility or lactic acid production in a Ringer-phosphate-glucose medium. It might therefore appear that sperm used the substrates of egg yolk solely by an oxidative process, but when stored in yolk-buffer, sperm remained equally motile under N_2 or air.

Arsenate, which accelerates the breakdown of hexose diphosphate, did not increase lactic acid production by spermatozoa and, in fact, concentrations of 0·01 M arsenate inhibited glycolysis. Similarly, reduced glutathione was without effect. (*See also* p. 138.)

Interrelation of Respiration and Glycolysis

MacLeod's work, demonstrating the predominance of glycolysis and the relative deficiency of respiration in the metabolism of human sperm, coupled with the fact that these cells retain maximal motility under anaerobic conditions, indicates that respiration is secondary and not an essential part of the metabolic function. Similar conclusions were reached for the sperm of the bull, dog and guinea-pig, in spite of the higher respiration rate of these cells in relation to those of human sperm (Redenz, 1933 ; Ivanov, 1931). Šergin (1935), however, states that experiments with boar sperm demonstrate that though glycolysis is absent the respiratory metabolism is very active.

The specific characteristics of metabolism of mammalian sperm apparently arise during the process of maturation in the epididymis, for Redenz has shown that while sperm from the rete testis have a relatively intense respiration compared with glycolysis, sperm from the tail of the epididymis have an intense glycolysis and weak respiration.

Lardy and Phillips (1941*a*, *b*, *c*) found that spermatozoa separated from semen were able to maintain motility in Ringer-phosphate solution only in the presence of oxygen. There was practically no motility in a nitrogen atmosphere, but with glucose added, motility was maintained as long as 3 hours in both air and nitrogen atmospheres. Thus, in absence of sugars, spermatozoa remain motile only in presence of oxygen. Addition of glucose prolonged aerobic motility and promoted anaerobic motility. Only sugars which sperm could catabolise to lactic acid (maltose, fructose and mannose, but not galactose and sucrose) were effective in maintaining motility. During storage inorganic P remains normal, acid-soluble P and ester P increase, and lipid P decreases ; this last change did not occur if the spermatozoa were previously heated to 80° C. The decrease in lipid P paralleled the decrease in motility. Lardy and Phillips (1941*b*) conclude that the energy for motility is obtained from the oxidation of intracellular substances (phospholipids) or from glycolysis. The anaerobic mechanism is sufficient when sugar is available and lessens the demand on the oxidative mechanism.

For ram sperm a correlation has been found between motility and glycolysis (Comstock, 1939) and between motility and respiratory activity (Chang and Walton, 1940). Comstock (1940) obtained a correlation of 0·88 between respiration and glycolysis rates. The data indicate that respiration and glycolysis rates are about equally related to viability. The experiments of Moore and Mayer (1941) indicate that a mechanism other than carbohydrate breakdown can provide the energy for motility.

Hydrogen-ion Concentration

The increase in the acidity of semen during storage is the result of the accumulation of acid metabolic products. Glycolysis, with the formation of lactic acid, has been noted in the semen of the bull and ram and the resultant acid *p*H is probably largely due to the lactic acid. Šergin (1935) stated that the shift towards acidity occurs at the expense of glycolysis and Moore and Mayer (1941) regarded lactic acid as the chief if not the only constituent. Change in *p*H may therefore serve to some extent as a measure of metabolic activity (Anderson, 1942*a* and *c*).

Moore, Mayer, and McKenzie (1940) reported the effect of *p*H on motility and metabolism of ram semen. They recommend that a *p*H determination should accompany every rating of motility of ram semen, unless the rating has been secured within 30 minutes of ejaculation and unless the semen has been buffered before the motility rating. Moore and Mayer (1941) conclude that the factor which influences motility and glycolysis in ram semen is the degree of acidity of the medium surrounding the sperm cells. No detectable changes occur in motility until the *p*H reaches a value of 6·0 or lower, and motility ceases at *p*H values of 5·5 to 5·3. It would appear that added sugar would shorten the duration of motility in ram semen unless proper precautions were taken to control the production of acidic metabolic products by the addition of a suitable buffer. The buffering capacity of the semen together with added buffer should be sufficient to control the acidity developed by glycolysis of the sugar normally present in the semen, in addition to that resulting from the metabolism of the added sugar.

Motility of semen of a rating of 2·5 and a *p*H of 5·5 after $3\frac{1}{2}$ hours at a temperature of 22·5° C.

could, by addition of an approximately equal volume of M/15 disodium phosphate, be restored to its original rating of 5 and continue at this rating for 20 hours, thereafter decreasing slowly to an 0 rating in the following 10 hours. In contrast, the original untreated semen continued to decrease to a rating of 0 at the fifth hour ; but if a drop of non-motile untreated semen was mixed with a drop of disodium phosphate numerous sperm were reactivated. Although the immobilisation of ram sperm by increased acidity is reversible, the degree of reversibility decreases with time.

During the period of 20 hours for which the motility, after removal of the effects of low *p*H by addition of basic phosphate, remained at the original rate of the sample, no sugar was present, nor was any added, which indicates that some substance other than sugar furnished the energy for the spermatozoa for this period, together with a further period of 10 hours during which the motility declined and ceased.

Comparison of the curves of *p*H and lactic acid indicates their lack of parallelism during the stages of glycolysis when the buffer in the semen and possibly other factors influence the *p*H. The decrease in *p*H is, however, associated with an increase in lactic acid. In one example the cessation of *p*H changes occurred at a time when no further increase in the lactic acid was demonstrable. They concluded that the study of glycolysis at various temperatures indicates that the sugar in ram semen is metabolised to an acid end-product which affects the motility of the sperm through changes in the *p*H.

The *p*H of the semen of the bull, stored undiluted and covered with liquid paraffin, has been studied by Anderson (1942*a* and *c*). The initial *p*H was of importance for the keeping quality of undiluted semen and the more acid the semen was on collection the better was the motility retained on storage at 8° to 10° C. Further, the initial *p*H of undiluted semen was associated with motility on storage whether the semen was diluted or not. Davis (1938) had noted that a high initial *p*H was not conducive to preservation and this observation was confirmed. None of the ejaculates with an initial *p*H of 6·9 or higher showed a high degree of motility after storage for 24 hours. However, Davis, Underbjerg and Williams (1940) stated that the initial variation of *p*H values appeared to have no significant effect upon motility after storage. Also initial variation in concentration and motility (50-100%) did not have an appreciable effect on the keeping quality of sperm.

Whatever the initial *p*H of the semen, the *p*H during storage may either decrease, increase for 24 hours and then decrease, or increase for 48 hours or longer (Anderson). Motility was best retained when the *p*H decreased steadily throughout the period of storage. When the *p*H increased for 24 hours or longer the motility was not so well retained. The more acid the semen was on collection and the less it moved in an alkaline direction during storage, the better was the motility retained. A move in the alkaline direction was more harmful for ejaculates with a high initial *p*H than for those with a low initial *p*H. The initial *p*H and the degree and direction of the change in *p*H during storage were therefore of primary importance for the retention of high motility in stored undiluted semen.

It appears that a change in *p*H in an alkaline direction is affected to some extent by the initial *p*H. Below an initial *p*H of 6·50 a higher percentage, and above 6·71 a smaller percentage of ejaculates decreased in *p*H during storage. Likewise, below 6·50 a smaller percentage of ejaculates increased in *p*H during storage. The more acid the *p*H on collection the less likely was a change in the alkaline direction during storage. According to Šergin (1935) the shift towards alkalinity shown by stallion and boar semen during storage is due to loss of CO_2 into the atmosphere, absence of lactic acid formation and the formation of ammonia.

The change in the *p*H of the semen of the bull after incubation for one hour at 37° C. was also studied (Anderson, 1944*a*). The decrease in *p*H was related to the initial *p*H, the motility and concentration of sperm ; significant differences were noted between bulls. The greater the decrease in *p*H the poorer was the motility after incubation. Motility was better maintained in semen diluted with egg-phosphate medium, compared with undiluted semen, which agreed with the smaller *p*H change in this medium. There was no evidence of a relationship between the *p*H change and the motility after incubation and the period for which a high motility was maintained during storage. The decrease in the *p*H of the semen of the bull after incubation was probably related to the metabolic activity of the sperm, though the degree of the change was probably affected to some extent by the buffering capacity of the semen.

Herman and Swanson (1941) determined the *p*H changes which occurred in semen of different bulls while stored, undiluted, at 40° F. The *p*H was determined before and after storage, which lasted on an average 128 hours (range 77 to 216 hours). The semen of 7 out of 11 bulls showed a rise in *p*H during this period, while 4 declined in *p*H. A study of these variations indicated that the

sperm were not killed in storage simply by the depression of the *p*H of the media. The stored semen was discarded at a given motility rating and the *p*H values at this time showed considerable variation.

Salisbury, Fuller, and Willett (1941) compared the *p*H of semen during storage in yolk-phosphate and yolk-citrate diluents at 5° C. and without mineral oil. The semen was divided into equal portions immediately after collection ; dilution was 1 part semen to 4 to 5 parts yolk-phosphate or yolk-citrate respectively. The rate of cooling was controlled and gradual. There was practically no difference between the average *p*H values of the two mixtures before or after storage for different intervals. Although the average values before storage were about the same, the *p*H values for the semen in the citrate buffer were more variable than those for the phosphate buffer, for their coefficients of variation were 1·12% and 0·73% respectively. This can be explained by the lower buffering capacity of the citrate diluent at these *p*H levels, with the result that the *p*H of semen, which was quite variable, would have a greater influence on the *p*H of the citrate than on that of the phosphate diluent. The decrease in *p*H after storage, for example for 6 days, was small, being 0·20 in both diluents.

Vitality in Female Genital Tract

The viability of sperm in the female genital tract, the transport of sperm and the passage of sperm through the cervix, uterus and uterine tube have been reviewed by Hartman (1939). The life of sperm in the vagina is generally regarded as short, the cervix being a more favourable site. According to Hartman, Hühner (1913) found that sperm live longest in the human cervix, and he attached considerable diagnostic importance to the microscopical examination of sperm recovered from the cervix. Living sperm have been found in the human cervix 80 hours post coitum (Cary, 1936) and in the uterine fundus 7 days post coitum. Day (1940*b*) noted that the sperm of one stallion lived for 72 hours in the reproductive tract of the mare, and probably some hours longer, as fertility was not low after this interval.

Sheep.—There is some difference of opinion as to the time taken by ram sperm to traverse the genital tract and reach the upper extremity of the Fallopian tubes. The times, following coitus, found by different workers are, (1) within 6 hours (Quinlan *et al.*, 1932), (2) about 5 hours (Kelley and Dumaresq, 1936), (3) about 5 hours (Green and Winters, 1935), (4) from 30 minutes to 7 hours 7 minutes (Phillips and Andrews, 1937), and (5) about 5 hours in Merino ewes (Kelley, 1937). Schott and Phillips (1941) observed that in 72 ewes of various breeds a time interval of 20 minutes after a normal service was usually sufficient for the sperm to reach the upper part of the Fallopian tube ; this was not influenced by the time of oestrus or ovulation.

Quinlan and co-workers (1932, 1933) studied the vitality of sperm of Merino rams in Merino ewes. In the vagina the majority of the sperm were non-motile after 12 hours. Living sperm were found in the cervix up to 48 hours after coitus, and it is believed that the cervix acts as a reservoir for sperm awaiting the availability of the ovum. These workers, assuming that the ovum is available for fertilisation between the 36th and the 40th hour (the interval after the onset of oestrus when ovulation was considered to occur), plus a few hours taken by the sperm to reach the Fallopian tubes, stated that the sperm are definitely capable of fertilisation for 36 to 42 hours after being deposited in the vagina. Green and Winters (1935) stated that sperm do not live more than about 24 hours in the genital tract. According to Polovceva *et al.* (1938), the average duration of survival of sperm in the female tract may be estimated at 34 to 36 hours, but in several instances eggs were fertilised which had ovulated 40 to 50 hours after insemination. Lopyrin and Loginova (1939) found that sperm retained their motility in the cranial portion of the genital tract of a ewe in heat (36 ewes) for 32 hours. Kelley (1937) found that the fertilising power of sperm from even the most fertile rams reached the threshold of infertility at approximately 34 hours after copulation. The fertilising power of the ejaculates from the majority of the Merino rams had a shorter duration than that of the ejaculates from Dorset rams. In his experiment the limit of fertilising power for Merino rams was 24 hours post-coitus. Further, there was evidence that the fertilising power of the ejaculates of Merino rams becomes reduced, if not lost, 10 hours after coitus. Anderson (1941*c*) found that sperm from Merino rams retained their fertilising power in the genital tract of the ewe for 22½ hours, the maximum period investigated. He did not observe any falling off in fertility within the limits of the experimental period according to the length of stay in the genital tract, but it is probable that as a rule fertility tends to diminish as indicated by Kelley. The vitality and fertilising capacity of sperm in the genital tract probably depend on a number of factors, which include the initial vitality and the capacity of retaining it, and the condition of the genital tract of the ewe.

Cattle.—Brewster, May, and Cole (1940) found that the minimum time required for sperm to reach the upper third of the Fallopian tube was $5\frac{1}{2}$ hours for mature cows and $4\frac{1}{4}$ hours for heifers. The length of the genital tract averaged $64{\cdot}95 \pm 2{\cdot}27$ cm. in cows and $52{\cdot}70 \pm 2{\cdot}13$ cm. in heifers. The rate of travel was not affected by age. Oestrus may affect it in heifers but not in cows. Experiments with dead sperm gave no indication that sperm travel is influenced by uterine contractions. In several cases disease abnormalities obstructed the travel of the sperm.

Šergin and co-workers (1940) observed that the conditions prevailing in the female genital tract of the cow, the predominantly alkaline reaction, and the high electro-conductivity of the secretion are such as to stimulate the activity of sperm, but to have an unfavourable effect on their survival, so that they die off rapidly in both the vagina and the uterus. In the cervix conditions are somewhat more favourable, *i.e.* a weakly acid reaction and absence of leucocytes ; here motility is reduced. In the cervix sperm can survive for up to 2 to 3 days, and a constant supply passes into the uterine horns. Counts of sperm in the cervix at various intervals after mating showed that the number reached a maximum in $2\frac{1}{2}$ to 3 hours ($157\text{-}168 \times 10^6$) ; at 5 hours it had fallen to about half this number and at 7 and 57 hours it was 30 and 11×10^6 respectively.

Morphology

The relation of sperm morphology to fertility was first demonstrated by W. W. Williams in 1920, and since then its importance has been amply proved. It is not on the whole possible to lay down definite limits, but the work on this subject does indicate a direct relation between the presence of abnormal spermatozoa and low fertility. It appears most reasonable, as McKenzie and Berliner stated, to believe that fertility depends on the absolute numerical proportion of abnormal spermatozoa in any one ejaculate. Also, according to Milovanov (1936) the appearance of pathological forms should be judged not only from the viewpoint of reduction of the total number of spermatozoa capable of fertilisation, but mainly as a symptom of the pathological condition of the genital apparatus of the male. In some cases a high number of abnormal spermatozoa may be found in semen which is otherwise normal, but in many instances an increase in abnormal forms is associated with other detrimental changes. Lagerlöf has pointed out that the detrimental agency which has affected spermatogenesis or the fully formed spermatozoa, may also have affected in some ways spermatozoa other than those which show morphological changes.

A somewhat different classification of abnormal or pathological types of spermatozoa is adopted by different workers, which is due, Milovanov (1936) considers, not only to differences in the actual material but also to insufficient knowledge of the subject. McKenzie and Berliner (1937) regard abnormal spermatozoa as either " malformations " or " deformations " according to the time and place of origin. Malformations are caused by disturbed spermatogenesis, either of a genetic nature or due to some environmental factors like heat, X-rays, seasonal variations, nutritional and endocrine deficiencies, that upset the spermatogenetic developmental stages in a cytological sense. Deformations occur when fully developed spermatozoa are partially destroyed through senescence after prolonged sexual rest, admixture of fluids from accessory sex glands, or a rise in body temperature too slight to affect spermatogenesis. Deformations were found in rams in March and April when spermatogenesis was still at a high level, and during the first stages of exposure to higher environmental temperatures before the malformed spermatozoa from the deranged testes became apparent. Malformations and deformations, they considered, could not be distinguished microscopically. Milovanov recognised two basic types of pathological changes in the spermatozoa, (1) primary changes occurring during spermatogenesis which indicate the existence of pathological processes in the germinal epithelium, and (2) secondary changes, due to prolonged retention of the spermatozoa in the genital organs of the male, owing to infrequent matings or to abnormal composition of the accessory secretions. Under primary changes, Milovanov placed giant and dwarf forms as well as all spermatozoa with changes in the head, and under secondary changes such abnormalities as fracture of the neck, acephaly, twisting and looping of the tail. The significance of other types of deformity, such as split or deformed tails, is not clear. The significance of the two different types of abnormalities for evaluation of fertility is very different, states Milovanov, for the presence of a large number of spermatozoa with fractured necks indicates incorrect husbandry and can be easily remedied, while head abnormalities have to be regarded as a sequel to serious disturbance of testicular function.

In relation to maturation of spermatozoa, Redenz (1924) considered that sperm acquire, during their passage through the epididymis, a thin protective membrane—the lipoid capsule—from the colloids of the epididymal secretions. Passage through the epididymal canal renders the sperm much

less sensitive to external media and their locomotory mechanism is not so easily disturbed, as shown by greater duration of movement. Contrary to this view, Young finds that older sperm, *e.g.* those from the tail of the epididymis as compared with those from the head, are less resistant than the younger sperm. Popa and Marza (1930) claimed to have demonstrated this film microscopically, for destruction of the capsule by sodium chloride liberates small lecithin globules which can be stained. Therefore according to Milovanov (1934) the state of the colloids of the lipoid capsule play an important part in determining the vitality of the spermatozoon and should be taken into consideration, both in studying the state of the spermatozoon in the organism, and particularly during various technical processes outside the organism such as sperm dilution and storage. Bernstein and Sokolova (1935) examined smears of semen undiluted and diluted with various salt solutions, after staining with Sudan III and scarlet red. Sudanophil granules were observed equally in both undiluted and diluted semen as well as in seminal fluid. These results do not confirm the view concerning the destructive effect of certain neutral salts on the lipoid capsule. The presence of a lipoid capsule can therefore be regarded only as a working hypothesis, they consider, for the only thing known about it is that its existence has not yet been proved.

Redenz stressed the importance of Retzius' protoplasmic drop which moves backwards from the head to the tail as the spermatozoon progresses along the tract. In the head of the epididymis the drop is attached to the neck of the spermatozoon while in the tail of the epididymis the drop is at the end of the middle piece. The drops also occur free in the semen. With frequent ejaculations at short intervals it was considered that the semen would contain more and more sperm with protoplasmic drops, indicating a drain upon sperm stored in the epididymis. Some workers believe that too frequent matings result in the ejaculation of immature sperm, and the presence of these drops has been associated with immaturity of sperm. Lagerlöf regards only those sperm with the drop on the neck as " immature ". Spermatozoa with the drop at the end of the middle piece apparently show normal vitality, but vitality is greatly reduced in sperm with neck drops.

It does not appear possible to attach great importance to these drops as indicators of physiological maturity for they occur even in the first ejaculate of the boar experiencing moderate sexual activity (Rodolfo, 1934*c*) and a large number of matings in a short time does not cause an increase in the number of " immature " spermatozoa (Kirillov, 1933-34 ; Gunn *et al.*, 1942). Selivanova (cited by Milovanov, 1936) found that the drops usually disappear under the influence of the secretions of the accessory glands. Their origin is no doubt related to the development of the sperm. A good account of spermatogenesis is given by Maximow and Bloom (1938).

From Lagerlöf's work on the bull it appears that the presence of more than 2% to 3% of " immature " sperm indicates pathological changes in the genital organs and reduced fertility. Gunn and co-workers agree with Lagerlöf that protoplasmic drops result from disturbance of spermatogenesis and not from failure of their removal during transit through the epididymis, for they observed them in sections of the rete testis, in the epididymis, and in ejaculated semen of abnormal rams. Also, they have only been found as late results of acute degenerative changes, or in the slow recovery stage, or in chronic degeneration. Spermatozoa with attached drops are therefore considered developmentally imperfect.

In the ram, Green (1940*a*) has studied the sperm membrane and the use of aceto-carmine technique and dark field illumination is suggested for the evaluation of sperm quality.

Lasley, Easley, and McKenzie (1942) devised a staining method for the differentiation of live and dead sperm based on the fact that in a mixture of sperm from several rams the former did not stain, whereas dead sperm, or sperm that were inactivated, stained with a mixture of equal parts of two solutions, one a 2% solution of water-soluble eosin and M/8 phosphate buffer, the other equal parts of opal blue and M/8 phosphate buffer.

MacLeod (1942) applied this technique to human sperm and found that the staining properties of the cells are reversible and cannot be correlated with the viability of the sperm in so far as motility is used as a criterion of viability.

No changes were observed in the morphology of bull sperm during storage from 72 to 360 hours (average 146 hours ; Herman and Swanson, 1941).

Fertility

Walton (1938*b*) has discussed the quantitative basis of fertility; in general, the principal determining factors are (1) the number of sperm, (2) the viability of sperm in the female genital tract, and (3) the time interval between copulation and ovulation. These factors vary considerably

in different species. The chances of fertility will be greater when there is a short interval between copulation and ovulation.

Walton has expressed graphically (Fig. 5) the quantitative relationship between the time of ovulation, the distribution of sperm at the Fallopian tubes and fertility. He writes : " The curves represent the distribution of spermatozoa at the site of fertilisation at various times after copulation. The first curve (N) represents the distribution resulting from a normal ejaculation. There is at the beginning a period without spermatozoa representing the time taken for the spermatozoa to ascend the tract. These, however, begin to arrive about 2 hours after copulation and the number increases rapidly until it reaches the minimum to ensure fertilisation. Then the number increases to a maximum and afterwards declines as the spermatozoa die off, until the number again falls below the minimum. The part of the curve above the minimum line represents the duration of the period of fertility and if ovulation occurs within this period, as at A, the eggs will be fertilised. If ovulation occurs later as at B or C the eggs will not be fertilised and sterility will result. Now, compare the result of altering the number of spermatozoa. Let us assume that only $\frac{1}{4}$ of the normal number of spermatozoa are present, as in curve N/4. It will then be apparent that although some spermatozoa reach the Fallopian

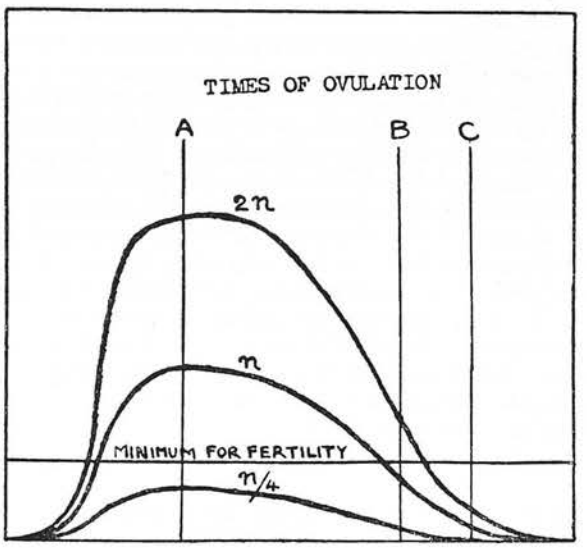


FIG. 5.—Relation between time of ovulation, distribution of sperm at Fallopian tube and fertility. (*From Walton*, 1938*b*)

tubes, they do not do so in sufficient number to effect fertilisation and the mating will be sterile. On the other hand, if the number of spermatozoa is twice the normal, as in curve 2N, twice the number of spermatozoa will be present throughout the whole period. The result will be an extension of the period of fertility as represented by the part of the curve above the minimum line. Hence ovulation at the time B will in this case be fertile, where it would be sterile if only the normal number of spermatozoa were present. Ovulation at C will, however, still be infertile.

" These curves therefore explain how the number of spermatozoa in the ejaculate may determine the duration of fertility. We can also see how the viability of the spermatozoa would affect the result, since with an increased duration of life in the female tract each curve instead of declining would be prolonged and cover a longer period of fertility."

It may be assumed, Walton stated, that the condition in the cow is represented in the diagram by ovulation occurring towards the end of the fertile period. In the cow there is therefore a critical period where the difference between fertility and sterility may be determined by the number of sperm in the ejaculate or by the viability of the sperm. From the results of Walton and Edwards (1938) which showed some correlation between fertility and the number of sperm, but an even closer correlation between fertility and the activity of the sperm, Walton concludes that even under normal breeding conditions fertility is not an " all or none " reaction, but a quantitative reaction in which the probability of fertilisation depends on the number and viability of the spermatozoa.

In the mare ovulation usually occurs towards the end of a long and variable heat period. There

may therefore be quite a long interval between copulation and ovulation. Hence low fertility would therefore be expected with mating early in heat. Hammond (1938) has shown that service more than 7 days from the end of heat is infertile, and that the chances of fertility increase as mating is made nearer the time of ovulation. Walton pointed out that ovulation may occur within or without the fertile period, depending upon the time of mating. He states " There must, however, be many cases when the timing relationships are critical and here the determining factors between fertility and sterility will be the number and viability of the spermatozoa."

Milovanov (1936, 1937, 1938) evolved a formula expressing quantitatively the relationship between number and resistance of sperm and fertility (*see* p. 17). Walton commented that clearly this formula introduces a large number of arbitrary constants and is mainly of empirical value. " Nevertheless it does," he stated, " introduce semi-quantitative expressions for the 3 main factors which must influence the probability of fertilisation, namely (1) the number of spermatozoa introduced, (2) the quality of the spermatozoa, and (3) the conditions favourable or otherwise to the ascent of the spermatozoa in the female tract."

McClean and Rowlands (1942) have indicated the possible rôle of hyaluronidase in fertilisation. Enormous numbers of sperm are produced by males, and any considerable reduction in the number causes sterility. The intromission of a large number of sperm is clearly required to ensure that even a single sperm shall enter the egg. The purpose of a large number of sperm in the immediate vicinity of the egg may well be, McClean and Rowlands suggest, the production of a concentration of hyaluronidase sufficient to liquify the hyaluronic acid gel and so allow penetration of the egg by the single effective sperm. It is possible also, that a deficiency of the enzyme may cause certain types of sterility for which there has been, hitherto, no obvious explanation. (*See also* p. 138.)

It is obviously to the characteristics of the spermatozoa themselves that one must look for a criterion or criteria of fertilisation, though other characteristics of semen such as the *p*H may provide useful auxiliary evidence. Recent work indicates that the measurement of metabolic activity, such as the respiration rate and glycolysis, hold considerable promise and are likely to be found of increasing value as the conditions which govern them are more fully elucidated. So far, however, it is doubtful if they can serve alone in all cases. Spermatozoa must be motile to reach the Fallopian tubes and sperm may show considerable metabolic activity and yet be poor in motility and other respects. It seems well, therefore, in the present state of our knowledge, to maintain a cautious attitude to the diagnosis of male fertility and to base one's estimate on several characteristics rather than on one.

(*See* discussions on bull, p. 9 and ram, p. 16.)

CHAPTER 6. STORAGE AND TRANSPORT OF SEMEN

Principles

Milovanov (1934) considers that death of sperm outside the body is due to one of three causes, (1) destruction of the spermatozoon itself, (2) expenditure of nutritive material, and (3) auto-intoxication by metabolic products. The cells themselves undergo senescence and their resistance decreases. The medium also undergoes changes ; glycolysis occurs with decrease of glucose, lactic acid accumulates, and there is uptake of oxygen and formation of CO_2. Storage of sperm outside the body involves bringing the sperm into a reversible state of activity, to slow down the metabolic processes and delay senescence. But since sperm in this state, termed " anabiotic state " by Milovanov, is still living, it is necessary to ensure a supply of energy material and also to render the metabolic products harmless. Conditions in the epididymis are the most favourable for continued viability of sperm, and Milovanov considers that the aim in storage is to reproduce *in vitro* the anabiotic conditions which obtain in the epididymis. This is easier with the semen of the ram and to some extent with that of the bull, in which the changes in the epididymal content that take place at ejaculation are slight, than with the semen of the stallion, boar, dog, and rabbit, which ejaculate a large amount of accessory secretions.

In general, storage of sperm is therefore based on (1) the gradual lowering of the temperature of the sperm to a level at which its functional activity is reduced to a low but reversible state, (2) addition to the semen of various substances which supply nutrients and control to some extent the metabolic reactions, and (3) removal of accessory secretions and concentration of sperm in a smaller volume of suitable diluent in the case of semen of the stallion and boar.

The first consideration for successful storage is to use only the highest quality semen. The better the quality on collection the better will the sperm retain motility on storage. One of the main problems

is the estimation of the potential survival capacity of sperm at the time of collection. Davis (1938) found that samples of bull semen having a high concentration of sperm kept the best. This has also been observed by the writer. Davis also noted that semen collected with the artificial vagina survived better than that collected by other methods. Herman and Swanson (1941) observed that the initial motility can be used for predicting the life of a semen sample ; if initial motility is very low, the duration of motility will be short. This, however, is of general value only, for semen of high motility initially shows considerable variation in the duration of high motility on storage. The initial *p*H of semen is also of general value for this purpose. A suspension of sperm is not a homogeneous stable population ; it is composed of a heterogeneous population of cells of various ages, activities, and powers of resistance (Chang and Walton). Seminal fluid is not always of uniform composition. Semen is also influenced by breed, individuality, age, body condition, season, nutrition, disease, sexual work, and so on.

No doubt much of the variation in the keeping qualities of stored sperm observed in earlier work was due to insufficient knowledge of the harmful effects of temperature shock. The requisite temperature conditions and the effect of various types of diluents and nutrients for survival of sperm have already been discussed (pp. 64-74). It is clear that diluents and nutrients hitherto used have fallen far short of providing the necessary conditions for prolonged viability of sperm *in vitro*. A very considerable improvement has been achieved by the use of embryonic chick tissue extract (Frank, Smith, and Eichorn, 1941).

Hronopulo (1940) attempted to control auto-intoxication and exhaustion of energy resources by the use of dialysis. Ram sperm was placed in a small inverted glass funnel with a cellophane membrane stretched across the mouth ; the funnel was immersed in a wide jar containing dialysing solution. The best results were obtained with a dialysing solution consisting of 40 ml. phosphate buffer, 30 ml. glycocoll, and 30 ml. glucose ; the optimum *p*H was 7·5. The surface area of the cellophane membrane influences survival, greater area (within limits) ensuring a more rapid diffusion of H ions between the sperm and the solution. A layer of vaseline (liquid paraffin) had a beneficial effect. The introduction of Ca, Mg, or lactate, or aeration [?] had no effect. The optimum volume was 100 ml. Activity was retained for 4 to 5 days, remaining at a high level (up to 0·7) for 2 days.

Walton (1933) recommends protecting the semen against rapid gaseous exchange by covering it with a layer of medicinal paraffin oil. Its effect is probably principally due to the retention of CO_2 (the writer has observed that, on storage, semen covered with liquid paraffin becomes more acid than uncovered semen), which if allowed to escape renders it unfavourably alkaline ; there may also be an effect due to ingress of oxygen. Walton concluded that protection of semen against exposure to the air may be advantageous, but that further investigation was required. Recently Steensma (1938) and Šergin (1939) have indicated that this procedure is not necessary and that sperm live longer with free access to oxygen. Salisbury and Asdell (1939) state that covering the semen with mineral oil is beneficial for the first one or two days, but for longer periods of storage there is some indication that the storage temperature has an influence on the desirability of covering the sample.

Temperature Control and Packing

Milovanov (1934) recommended placing semen in narrow test tubes and covering it with a layer of liquid paraffin (Walton) at least 1 cm. thick, the tubes being stoppered so as not to leave any air under the cork. To guard against temperature shock, the test tubes must be wrapped in cotton wool and, to prevent penetration of water, placed in other larger ones. Similar methods are used by other workers. Phillips, Schott, and Gildow (1938) placed ram semen in small vials under a layer of mineral oil ; these were wrapped in cotton wool and placed in larger vials, which were then packed in vacuum containers with ice and cotton wool ; after transit they were kept in a refrigerator until required. Under these conditions it was found that the temperature had been reduced too quickly and that the low temperatue (5° C.) was not sufficiently evenly maintained. A suggested improvement was gradual cooling by changing the semen from one container to another, each cooler than the previous, prior to packing in the vacuum flask.

For storage and transport of bull and ram sperm Walton (1942) recommends the use of a vacuum collecting flask, to prevent rapid cooling. The flask with the semen is then placed in a series of cool chambers or thermos jars with water at the required temperatures. It should be kept for half an hour at 15° C., half an hour at 10° C., and one hour at 5° C. The semen is then placed in stoppered test tubes and stored or transported in a thermos flask with ice. Herman and Swanson used the following method for bull semen : the vials containing semen were wrapped with several thickness of paper towelling, covered with a rubber finger stall and placed in a thermos of water at

40° to 50° F., where they were allowed to cool gradually to the temperature of the water. The small vials of semen were then placed in a tray filled with water at 40° F. and put in a refrigerator, which maintained a temperature of 40° F. with a range from 36° to 45° F.

There is general agreement in American artificial breeding associations that semen should be cooled gradually, but that no definite procedure is necessary to warm it before use. In these associations various means are used to cool the semen gradually ; wrapping tubes in flannel or other material ; placing specimen tubes in a jar of water large enough so that cooling takes place at a rate of 1° F. per minute when the sample is placed in a refrigerator; double walled test-tubes (Trimberger, 1942 ; Salisbury, 1941).

Salisbury (1941) stated that the improvement which has taken place in storage and transport of bull semen has been brought about by many factors—appropriate dilutors, proper cooling of semen, development of satisfactory shipping containers so as to control the temperature of semen in transit, development of time-saving devices which enable the operator of the laboratory to control, rapidly and to a large extent, the quality of the semen which is shipped.

Owing to the short life of horse sperm Walton does not recommend transport except for quite short distances. The semen can be kept for some hours if protected with paraffin and slowly cooled to 10° C., but the fertilising capacity rapidly falls off. Improved laboratory methods are, however, available.

The packing of capsules with gelatinised sperm is done to prevent any too sudden cooling or wetting in the event of rain (Milovanov, 1938). Taking into account the convenience of capsules for small herds each capsule should be packed separately. They were packed in cheap, grey cotton wool enclosed in waxed paper. For transport an ordinary laboratory thermostat with double walls, in which the capsules were placed vertically, was employed. The space between the walls was filled with water at 10° C. A range of temperature of 7° C. to 18° C. in the thermostat during transport did not affect the percentage of fertilisation.

Lambert and McKenzie (1940) give the following method for handling and transporting semen. When inseminations are to be made within 2 hours, it is sufficient to place the semen in a small stoppered vial, which may be left at room temperature until the semen is used, but should be placed in a closed container or in a dark place and under no conditions exposed to direct sunlight. When the semen is to be kept for longer periods, additional precautions must be taken. The semen immediately after collection should be placed in a clean vial, covered with a layer of high-grade neutral paraffin oil up to the cork, leaving no air space in the vial. The vial is then wrapped in 2 thicknesses of paper and set in the refrigerator, or the paper-wrapped vial may be covered with two rubber thumbstalls and dropped into a vacuum flask containing water at from 3° to 8° C. This allows gradual cooling to and maintenance at this temperature. Instead of using paraffin oil, paraffin wax plugs can be pressed into the vials down to the semen, and sealed with a few drops of melted paraffin wax. This method has been used successfully for bull, ram, and goat semen. Duarte (1940) found that the temperature decreased from 18°-25° to 0° C. in about an hour when the sperm tubes were wrapped in cotton-wool which was enclosed in a rubber bag and placed in a thermos with ice.

When semen is to be sent a long distance, more elaborate precautions are necessary. The vial with the semen is wrapped in a layer of cotton-wool, after which it is placed in a screw-top, watertight glass vial, which is, in turn, well wrapped in cotton-wool held in place by rubber bands. The package is then placed in a quart vacuum flask and tightly packed with chipped ice. To avoid danger of breakage a layer of cotton-wool should be placed in the bottom of the flask before packing with chipped ice, and another layer added before stoppering. The semen may be kept at a temperature below 10° C. for 30 hours if properly packed in this way.

When the semen is removed from the refrigerator or the vacuum flask it is best to raise the temperature gradually before inseminations are made. This may be done by letting the vial stand at room temperature for from 30 minutes to one hour, after which the vial may be set in tepid water (33° to 35° C.) for a few minutes.

Olive oil frozen to a semi-solid state appears to be the most promising medium for holding semen at the optimum low temperature long enough to permit its shipment to distant points, since it absorbs more heat and appears to be capable of holding a low temperature longer than water (U.S. Dept. Agric., 1939*b*).

Transport

Lambert and McKenzie (1940) remark that very careful co-ordination of effort is necessary between the persons shipping the semen and those doing the insemination. It is necessary to plan the shipments so that a minimum of time will elapse between shipping and insemination. The females

to be inseminated must be on heat and at the proper stage of heat, that is shortly before ovulation, if a high percentage of conceptions is to be obtained. A careful checking of the oestrous cycles of any females to be inseminated is therefore required so that plans may be made to have semen at the proper time during subsequent heat periods. It will also be necessary to perfect apparatus for holding the temperature of the semen within rather close limits (3° to 8° C.) for the interval of time required for shipment. These authors consider that, because of these difficulties, the transport of semen will probably be confined to the services of an outstanding sire that could not be obtained otherwise. The widespread development of air services would allow shipment of semen when the transport of either the sires or dams would be too costly or otherwise not feasible. Lambert and McKenzie state that its most effective use would seem to be at large experimental stations, on ranches, or in organised groups to spread the services of a sire over a large number of females.

Aerial transport has been used for distributing sperm among farms in Russia (Milovanov, 1938). To expedite transport and economise flying time, the sperm was usually dropped without landing. The required number of capsules was taken out of the thermostat, fixed together by rubber bands and provided with a vane made of newspaper, which ensured the horizontal orientation of the package when dropped. Packages were dropped from a height of 10 to 300 metres and in no case were the capsules injured or sperm rendered defective. To inform the plane as to the required number of capsules, each farm had a semicircular landing 3 metres in diameter, covered with coal or slag and surrounded by a whitewash border. Beside this on the ground was traced in whitewash the number of the farm. Figures measuring 30 to 50 cm. were made of wood and painted white, and every day after testing the females the required number was shown on the landing. The time between reception of sperm and insemination should not exceed half an hour.

Bull Semen

Storage

The use of undiluted semen for storage was recommended by Kufarev (1935), Weber (1936), and Komarov and Gladcinova (1937). Petrov and Schneerson (1933) recommended that semen should be kept undiluted under vaseline oil for 24 hours to produce rapid anabiosis and then diluted. Vorobjev and Schneerson (1933) found that with semen kept up to 30 hours undiluted at 8° to 12° C., the percentage of pregnancies was not reduced.

Hatziolos (1937) concluded that dilution (physiological salt solution, Ringer, Tyrode and Russian glucose-buffer solutions) offered no advantage for sperm storage. Undiluted semen at 0° to 6° C. kept better than diluted semen ; of 20 cows inseminated with semen stored for from 24 to 128 hours, only 2, inseminated with 24- and 48-hour semen respectively, became pregnant.

Delitz (1939) found that survival of bull sperm was greatest when diluted by 3 volumes of GPS-2, and was better at 6° C. than at 10° or 12° C. (maximal survival 142 hours). Tyrode solution gave maximal survival at the same dilution, while glucose-Tyrode gave a lower survival time (48 hours).

Bonadonna (1939*c*) stored bull semen after gradual cooling. No particular advantage was noted from adding glucose-phosphate, glucose-tartrate, or glucose-sulphate diluents (as also with sheep and goat semen). Sperm retained a motility of 5/5-3/5 for 200-287 hours. One specimen retained a motility of 3/5 for 455 hours. Henderson (1939), using semen diluted with SGC-2 and kept at 45° F., observed no difference in the conception rate of semen kept up to 30 hours. Burch (1939) found that semen stored for 24 hours at 42° F. compared favourably in fertility with fresh semen, but many samples were not good enough to use.

Herman and Swanson (1941) found that 3% and 5% glucose and Milovanov's SGC-2 were all detrimental to the sperm. More vigorous motility was maintained in the undiluted semen. The time of survival was nearly the same in the SGC-2 dilutor as in the undiluted semen, but the percentage of motile sperm was less.

Anderson (unpublished experiments) compared the effect of storing bull sperm undiluted or diluted $\times 2$ with the TGC diluent. The semen was stored at 7° C. to 10° C. ; the test tubes were wrapped in cotton wool but it is probable that the rate of cooling was not as gradual as subsequent work has shown to be necessary. The mean percentage motility was very similar in the 2 groups, but comparing specimens with a motility of 70% or more, the undiluted semen was superior ; at 24 hours the percentage of specimens of this motility was 74 in the undiluted group and 33 in the diluted group ; at 48 hours it had fallen to 34 and 10% respectively, while there was little difference at 72 hours. The sperm from individual bulls varied considerably in keeping qualities. The fact that this was shown with diluted as well as with undiluted semen points to a superior vitality in the

sperm of some bulls. There was also considerable variation in the keeping quality of individual specimens from the same bull; the best motility observed in any specimen was 50% at 144 hours and 20% at 168 hours.

Anderson (1938*a*) reported that thirteen cows were inseminated with semen stored 6 to 8 hours and 6 conceived (46%), 43 with semen stored 24 hours and 19 conceived (44%), and 2 out of 5 cows conceived with semen stored for 40 hours. Since then many cows have been inseminated with stored semen. Egg-yolk diluents have proved highly successful for this purpose (unpublished data). So far semen 5 days old is the oldest that has been used successfully, but semen has been stored for periods up to 72 hours with little difficulty and given insemination results equal to those from the use of fresh semen.

It was observed that the semen of individual bulls varied as to the effect of egg-yolk buffer (Herman and Swanson), which seemed to give little beneficial effect on motility in four bulls, while in two whose semen was thicker and more viscous on storage, it gave strikingly favourable results. Viscosity may therefore determine whether or not dilution will enhance the viability of semen on storage. Replacement of seminal fluid with egg-yolk-buffer diluent resulted in a high rating of motility for the first 3 days of storage. Storage of the concentrated, centrifuged, undiluted sperm was not beneficial.

Herman and Swanson performed 26 inseminations, which resulted in 15 conceptions (average of 1·73 per conception), with sperm stored from 4 to 198 hours (average 49 hours). The average motility rating was 3, and the range from 1 to 5. Only 2 conceptions were obtained from 4 inseminations with semen more than 100 hours old, but these results indicate that semen stored undiluted up to 100 hours and of good motility was effective in causing conception.

In a study of 931 cows artificially inseminated with semen, undiluted and diluted with fresh and stored egg-yolk buffer and autoclaved milk, Underbjerg, Davis, and Spangler (1942) observed that the treatment did not cause a significant lowering of the conception percentages as contrasted with controls. Despite the maintenance of sperm motility, stored groups showed a significant departure in conceptions from the fresh semen. Evidently the treatments had little beneficial effect on fertility, but other factors were in operation during storage which caused a loss of the fertilising capacity regardless of the retention of motility.

In co-operative artificial insemination of cattle, Hamilton and Symington (1939) obtained favourable results by keeping the sperm at 35° to 45° F.; the diluent then preferred was SGC-2; later (Hamilton, 1940) Phillip's egg-yolk diluent was used with successful results.

Trimberger (1942) obtained information on present-day methods from 64 artificial breeding associations in the U.S.A. Egg-yolk-phosphate and egg-yolk-citrate dilutors were used with about equally good results. About 40° F. was the common storage temperature after gradual cooling. Many associations successfully used semen (with 50% or more conceptions) that had been stored up to 72 hours. Salisbury (1941) states that semen as handled when artificial insemination was first used in New York could not be depended upon to maintain its fertility for more than 24 hours after collection. To-day as a routine practice semen is not only shipped many miles but is often used 4 days after collection and occasionally much longer.

In the first report on the egg-yolk-phosphate diluent Phillips (1939) recorded that by its use very active motility was maintained for from 120 to 150 hours and that pregnancy resulted with sperm stored for 120 hours; Phillips and Lardy (1940) reported pregnancies from semen stored in this manner for 180 hours. Willett, Fuller, and Salisbury (1940) found that yolk-phosphate diluent maintained the viability of the sperm at a higher level during storage compared with semen stored undiluted. Data on over 1400 inseminations in an artificial insemination association indicated that, when proper precautions are taken in handling the semen, it may be diluted and stored up to 4 days with results as satisfactory as those with semen used on the day of collection. Storage with egg-yolk-phosphate gave better results than dilution with the Russian diluent SGC-2. Swanson and Herman (1941*b*) found that yolk-phosphate benefited the motility of bull sperm for the first 100 hours, and if the semen was centrifuged, the seminal fluid being replaced immediately by 4 volumes of egg diluent, still greater motility was obtained. However, in both cases survival time after 5 days' storage was usually less than in good quality undiluted semen. The addition of glucose to centrifuged egg-yolk diluted semen had no effect.

Salisbury (1941) found that yolk-citrate and yolk-phosphate diluents equally preserved motility of spermatozoa stored under standard conditions for 2 and 4 days, cooling from room temperature to 5° C. at the rate of 5° C. per 10 minutes. When semen was stored for 6 days or more, the yolk-citrate was superior to the yolk-phosphate diluent. In insemination tests no significant difference in

fertility was found between semen stored up to 5 days in yolk-citrate and in yolk-phosphate diluents. The writer has obtained very similar results with these diluents and both are much superior to any others previously used.

Herman and Swanson (1941) noted that the greatest loss of motility in stored semen occurred during the first 24 hours. From 24 to 48 hours the decline in motility was very slight and from 48 to 168 hours it appeared to be roughly linear. Comparison of semen diluted with egg-yolk-buffer medium with similar samples stored undiluted showed that dilution caused the maintenance of more vigorous motility. This beneficial effect was observed up to the 5th day of storage. After the 5th day motility in the diluted semen dropped markedly while motility in the undiluted semen was maintained fairly uniformly with the result that it averaged higher than in the diluted semen. It would therefore appear that the use of this diluent would enhance the vitality of the sperm up to the 4th or 5th day, but for periods of longer storage it would give little benefit. (*See also* p. 138.)

Transport

In 1932 Milovanov stated that the distance to which bull semen was transported should not be more than a 2 hours' journey and not more than 6 hours should elapse between collection and insemination.

Bull semen collected in the Netherlands has been successfully used for insemination of cows in England (Edwards, Walton, and Siebenga, 1938). Lambert and McKenzie (1940) reported the sending of semen from Beltsville, Maryland, to Piran in the Province of Buenos Aires, with at least one successful impregnation. In this experiment 7 days elapsed between collection and insemination.

The Central New York Artificial Breeders' Co-operative, of which 18 of the 22 artificial co-operatives in New York are member units, supplies semen to all member units from bulls housed near Syracuse. In some cases the semen is sent 200 miles or more and the ejaculate collected one morning may not reach its destination until the next day.

Scorgie (1943) has sent samples of bull semen from Reading to Lancashire and the Isle of Man, and at least 4 cows have been successfully impregnated with semen collected 48 hours previously.

Ram Semen

Storage

Since glycolysis is so intense with ram sperm, and death of sperm even at low temperatures is due to the acid reaction, Milovanov recommended adding buffer phosphate (phosphate solution from the diluent GPS-2) at the rate of 0·2 ml. per 1 ml. semen.

Habibullin (1937) investigated the storage of ram semen diluted 1 : 1, 1 : 2, and 1 : 3, with glucose-phosphate diluent (GPS-2, Milovanov) and buffer phosphate. The tubes were wrapped in cotton-wool and gauze, placed in vials and kept in thermos flasks at 8° to 10° C. GPS-2 gave slightly better results than the phosphate buffer, *e.g.* after 12 hours' storage the rate of fertilisation was 77·6% compared with 69·2% for the phosphate buffer, though after 24 hours the difference had nearly disappeared. It was suggested that the less favourable effect of the phosphate buffer was partly due to insufficient glucose content. Sperm diluted up to 1 : 2 and stored for 6 hours gave comparable results to fresh sperm (73-92% lambing); after 24 hours it gave a fertilisation rate of 54-64%. Sperm undiluted and diluted 1 : 3 showed a drop after 12 hours. Habibullin concluded that undiluted semen after 6 hours' storage can be widely used in breeding practice, but for longer storage (12 to 24 hours) semen should be diluted with GPS-2.

Milovanov and Habibullin (1933) found that ram sperm survived at least 9 days when stored at 1° to 2° C., and Gunn (1936) that the average life of all samples stored at 4° C. in small tubes was about 12 days. When special care was taken to exclude the watery part, and when collected in an ideal way and suitably treated, the life of sperm at 4° C. was extended to 40-65 days.

Winters (1936, cited by Lambert and McKenzie) reported 2 successful impregnations with ram sperm that had been kept in the laboratory for 6 days, 3 with sperm kept 5 days, several with 3-day-old samples, while many were obtained with 1- and 2-day samples.

Transport

Walton and Prawocheński (1936) sent ram semen from England to Poland, and obtained successful impregnations 51 hours after collection. Phillips, Schott, Terrill, and Gildow (1939) reported that during 1936, 1937, and 1938, ewes were inseminated with semen sent by air express from Dubois, Idaho, to Moscow, Idaho (700 miles), from Beltsville, Maryland, to Moscow (2600 miles) and in both

directions between Beltsville and Dubois (2250 miles). In the first 2 seasons ewes were inseminated once only and at varying times during the oestrous period. In the third season they were inseminated 12 hours after they were first observed in heat and again at 12 hour intervals as long as oestrus continued, or until the semen supply was exhausted. Of the 309 heat periods at which inseminations were made 31 resulted in pregnancies and 36 lambs were produced. The best results were obtained with semen from 2 rams sent from Dubois to Beltsville ; inseminations were made at 53 heat periods and 13 pregnancies resulted. The oldest semen used successfully was 115 hours. No consistent trends were found in the proportions of success obtained with semen in the various ages from 22 to 115 hours. In the 1937 experiments from Beltsville to Moscow (Phillips, Schott, and Gildow, 1938) of 17 samples only 12 arrived in a satisfactory state and the age of the sperm when used was 31·5 to 200 hours. There were 5 pregnancies in 53 ewes (60 inseminations) and they were all from samples under 72 hours (37 inseminations, *i.e.* 7·4 inseminations per pregnancy). Four of the pregnancies resulted from 17 inseminations with sperm under 48 hours old, *i.e.* 4·2 per pregnancy. The average number of natural matings per pregnancy required by the same rams was 2·6. The results indicate that improved technique is required where storage periods of one day or more are necessary. There was wide variation in the quality of semen used. No pregnancies resulted from the semen of 7 rams. There is therefore definite need, the authors state, for more work on methods of evaluating semen. The various measures of semen quality (volume of ejaculate, sperm per cm.^{3}, abnormal sperm per 1000, motility at collection and on arrival at destination) were studied in relation to successful use of the semen and a consistent trend was observed in only one criterion, the motility score on arrival.

Milovanov (1938) studied the effect of time of packing and transport upon fertilisation in vaginal insemination of sheep with gelatinised sperm (diluted with GPS-2-G). Insemination up to 6 hours after collection gave satisfactory results, but results were inferior after 24 hours. Milovanov, Nagornyĭ, *et al.* (1939) investigated the transport of sperm over a distance of 50 to 80 km. A fertility rate of 80% to 90% was attained by the following technique : using sperm with high resistance only (not less than 5000), which could be obtained by an adequate diet ; maintaining a temperature of 15° to 20° C. in the thermos during transport and ensuring perfect thermo-insulation by packing in gauze and cotton-wool. Dilution with a buffer phosphate did not raise the rate of fertilisation, but caused higher mortality of sperm during transport. Testing of sperm resistance both before and after transport was recommended. For longer periods of transport a lower temperature is indicated. Băicoianu (1939) in one experiment sent ram sperm a distance of 608 km. and inseminated 14 Karakul ewes after an interval of 28 hours from the time of collection ; 3 lambed. In a second experiment the interval between collection and insemination was 27 hours ; 22 ewes were inseminated and 6 lambed. Hronopulo (1940) sent ram sperm from Askania Nova to collective farms of the Rostov district, a distance of 1000 km., the total interval between collection and insemination varying from 24 to 36 hours. Altogether 58 ejaculates were transported, and 37 were found fit for insemination. The rates of fertilisation were only 20% or less. It was concluded that, since activity of sperm at 38-40° C. cannot be taken as a sign of fertility, methods of storage should be assessed by the results of insemination and not merely by survival of sperm.

Stallion Semen

Storage

To avoid destruction of the lipoid capsule at low temperatures, two measures for strengthening the lipoid capsule of the sperm of the stallion, boar, and rabbit were considered necessary (Milovanov, 1934). This was done by reducing the electrolyte content 8 to 10 times, bringing it nearer to that of the epididymis, and by substituting sulphate and tartrate for chlorides. (The introduction of tannin was also helpful.) Both may be achieved, Milovanov states, by diluting 8 to 10 times with an appropriate fluid (*e.g.* TGH-5) followed by centrifuging. Under these conditions, the fall of temperature and acidification began to give an effect analogous to that obtained on sperm of other animals. Milovanov, Lihačev, and Ževanova (1939) have given further attention to this question. Stallion sperm in an undiluted ejaculate usually perishes in 3 to 4 hours, which renders storage and transport impossible. The rapid death of the sperm is caused by (1) unfavourable *p*H conditions, leading to continuous stimulation, absence of natural anabiosis, and disintegration of the lipoid capsule ; and (2) lack of glucose, which results in a rapid expenditure of the slight energy resources (presumably lipoidal). Any technique of storage must therefore overcome these harmful influences. The necessary conditions of (*a*) dilution with a glucose diluent, containing small amounts of anions with negative lyotropic activity, (*b*) the regulation of the oxidation-reduction process, in order to create a state of anabiosis,

and to replace oxidation by the more profitable process of reduction by excluding air, and (*c*) an appropriate temperature, were fulfilled by the use of the diluent TGH-6 and storage at 8° to 10° C. Survival was increased 11 to 12 times compared with undiluted sperm. While undiluted sperm survived equally badly at all degrees of aeration, with sperm diluted with this anabiotic diluent, the period of survival was inversely proportional to the aeration. It was accordingly concluded that in sperm transport, narrow sperm containers and test tubes should be used and that sperm should not be shaken up. Thirty mares were inseminated with sperm stored 6 to 8 hours and another 30 served as controls. The rate of conception in the 2 groups was 66·7% and 70% respectively.

Walton (1938*a*) obtained fertile inseminations with sperm, twice diluted, centrifuged, and stored for 24 hours at 0° C., but not with sperm similarly stored for 48 hours. Lihačev (1939) diluted semen from one stallion ×4 or ×5 with Milovanov's tartrate diluent (prepared daily) and stored it at 8° to 9° C. Of 30 mares inseminated with sperm stored 6 to 8 hours, 20 became pregnant (8 required 3 to 5 inseminations). Hence stallion sperm can be stored for this period without impairing its fertilising properties ; it should be of sufficient density and should be cooled gradually. Paršutin (1939) experimented on stallion semen with a view to (*a*) preparing diluents which would neutralise the destructive action of the secretions of the accessory glands, and (*b*) introducing into the semen nutritive carbohydrates in order to substitute glycolysis for the processes of respiration and oxidation of non-carbohydrates (including the lipoid capsule of the sperm). Tests were made with 7·2% glucose solution with isotonic concentrations of sulphate, tartrate, acetate, 5% citrate, and 0·95% NaCl, varying the proportion of electrolytes. Tartrate and citrate had no marked effect while acetate and chloride, even in small quantities, decreased duration of sperm survival. Addition of 1·6 to 3·2% sodium sulphate gave a slightly increased survival. At 10-13° C. survival was nearly double that at 18–20° C. He concluded that dilution increased by several times the duration of survival of sperm compared with undiluted samples. The introduction of a small amount of isotonic glucose had little effect. The good results obtained on a four-fold dilution with a 7·2% glucose solution confirm the view that the death of sperm in undiluted semen is chiefly due to the destruction of the capsule by chlorides. It was considered that the proportion of sulphates in existing glucose-sulphate diluents should be reduced.

McKenzie, Lasley, and Phillips (1939) reported the successful impregnation of 2 mares with semen stored for 20 hours, in one case after transport of the semen from a considerable distance. They described in detail the method used in treating the sperm-concentrated fraction of the semen.

Berliner, Cowart, and Pharis (1940) obtained the best results in the preservation of motility of stallion and jack sperm by storage of diluted semen with gradual cooling to 40° to 45° F. Conception was obtained with 24-hour sperm.

Soliven and Gonzaga (1940) studied the effect of adding stallion semen to various diluents, stored at 2° to 5° C. or 8° to 10° C. After diluting 1 : 4 or 1 : 5 with Russian tartrate or sulphate diluents, all sperm were generally dead within 24 hours. The Cambridge method was not successful, nor was storage with tartrate diluent in a sealed ampoule. Anabiosis was impossible using CO_2 gas, 0·1 and 0·01 KCN solutions, and 0·5 and 1·0% Na_2CO_3 solutions. Of various proteins, protein derivatives and substances containing protein added to the diluent, none had any favourable effect on longevity of sperm except forms of lecithin and a water soluble extract of yolk of hen or duck eggs. Decrease in the percentage of motile sperm was always accompanied by a decrease in *p*H, complete loss of motility occurring at *p*H 4·6 to 4·7, even with storage at low temperature. Vitamins A, B, C, and D added in combination were favourable, but none of them had any favourable influence on longevity when added singly. Preliminary studies by Gonzaga and Valenzuela (1934) were more encouraging.

Semen of Other Animals

Boar.—Semen has been successfully stored by removing the gelatinous lumps and then adding an equal volume of Milovanov's diluent. McKenzie, Lasley, and Phillips (1939) used semen from boars of proved fertility to inseminate 63 Poland China gilts, 24 of which became pregnant, storage times of 12, 24, and 56 hours giving positive results. The percentage of pregnancies was significantly increased with 2 or more inseminations per heat period. (*See also* p. 139.)

Dog.—Bederke (1933) found that dilution of dog semen with physiological solution reduced the time of survival to 12 to 15 hours compared with 21 hours for the normal. Dilution with dog serum reduced the time of survival by half. Dextrose solution, alone or with sodium phosphate buffer, had an unfavourable influence.

According to Freiberg (1935) the 2nd fraction of the ejaculate, *i.e.* the sperm-containing fraction, can be kept undiluted up to 20 to 25 hours, whereas in the 3rd, *i.e.* the prostatic, fraction the survival is only 3 to 7 hours.

Rabbit.—One of the earlier and successful transport experiments was that of Walton in 1926, when rabbit sperm, sent by post from Cambridge to Edinburgh, was used for insemination 46 to 49 hours after despatch.

Cock.—Grodziński and Marchlewski (1935) found that in samples of cock semen diluted $\times 10$ or $\times 20$, motility (even progressive motion) was retained for a considerable time (in some cases over 120 hours), while in dilutions of 1 : 40 to 1 : 320, no progressive motion was observed and oscillatory motion ceased rapidly. Tests with other diluents indicated that the most favourable was blood serum, followed, in the order given, by centrifuged extract of crushed whole chick embryos, egg albumen, Tyrode fluid, and blood plasma mixed with semen coagulates. The optimum *p*H was 7·2 to 8·0. Semen diluted with albumen retained its motility longest at 16° C., while in other media the optimum was at 2° C.

CHAPTER 7. EXAMINATION OF SEMEN

Semen should preferably be collected with the artificial vagina or, in the ram, with the electrical stimulation method as described in Chapter 10. No single method is entirely adequate for the evaluation of semen, and so a combination of methods is therefore employed. The following points are included in the examination of semen : (1) appearance, (2) volume, (3) motility of sperm, (4) number of sperm per mm.^{3} and per ejaculate, (5) *p*H of semen and the *p*H change on incubation, (6) proportion of abnormal spermatozoa, (7) respiration rate of sperm, (8) resistance of sperm, (9) longevity of sperm, and (10) freedom of semen from bacteria, parasites and cells, which indicate a pathological condition of the genital organs of the male. As complete an examination as possible of the semen should be made, and when males are used intensively such examinations should be made periodically. In males whose semen has been found of high quality, its fitness for artificial insemination can, on the whole, be determined from the motility and concentration of the sperm. A clinical examination of the genital organs of breeding males should also be made.

As semen is often of poorer quality after a long sexual rest and in certain seasons of the year, as for example with rams in the summer months, ejaculates collected under these conditions may not be typical of the semen production of the male in question. When testing males after a long sexual rest, therefore, the first ejaculate, or the first one or two, should be regarded as possibly atypical and the estimate based on subsequent ejaculates. Also, tests should be performed as far as possible under optimum seasonal conditions. Semen from different species differs considerably, so when examining an ejaculate, the typical characteristics of the particular species must be kept in mind.

An examination based on one ejaculate refers to that ejaculate only. It is therefore desirable to base the estimate of fertility on examination of as many ejaculates as possible, in order to obtain the average and range of variation in semen characteristics for the particular male. As considerable time is required for the collection of a number of ejaculates, it may be said that a single good specimen probably indicates that the male is of good fertility, but a poor ejaculate by no means condemns the male. A succession of poor ejaculates produced over a period of time would, however, indicate a less efficient breeder. In the present state of our knowledge, it is only in the extreme cases in which sperm are absent or dead that sterility can safely be prophesied. In other cases, it can only be said that in certain circumstances fertility will probably be " reduced " or " low."

The reproductive capacity of a male may be measured not on a single ejaculate, but on the total ejaculate produced in a given interval of time upon several different occasions (McKenzie and Berliner, 1937).

Physical Properties

The general appearance of the semen, the colour, consistency, opaqueness, and any abnormal qualities are noted. The colour and thickness vary with the concentration of the spermatozoa ; the higher the concentration the whiter, or more creamy, and the thicker is the semen. When spermatozoa are few or absent the ejaculate is usually thin and watery. Ram semen is much thicker and more creamy than bull semen. The colour may be changed by the admixture of urine, as may happen in the ram when the electrical stimulation method of collection is used, and by urine or faeces in the fowl. The presence of blood causes a reddish or pinkish colour. Floccules, particularly noticeable when the semen is diluted, may be noted in abnormal ejaculates from the bull. A gelatinous coagulate is normal in the semen of the stallion and the boar. The viscosity of semen can be determined by the method of Denning and Watson (1906). Normal semen has little or no smell, but in abnormal conditions of the male genital organs it may have a highly offensive smell.

The volume is determined in a pipette graduated to the tip, or in a graduated cylinder, collecting bottle, or tube.

Motility

The essential points to be observed in the estimation of motility are: (1) examination as soon as possible after the collection of semen, (2) maintenance of optimum temperature, and (3) prevention of drying of the semen. If the semen is not examined immediately after collection a fall in temperature may reduce the activity of the sperm. The semen must not at any time be exposed to the sun.

The ejaculate is examined microscopically. A small drop of semen is placed on a cover slip with a pipette and a hollow-ground slide inverted over the cover slip. The slide is then turned over and placed on the microscope stage. If cover slips and hollow-ground slides are not available, a drop of semen can be placed on an ordinary glass slide and a drop of medicinal paraffin placed on the semen to prevent it drying up, but for routine practice it is recommended that hollow-ground slides be used. An alternative method for use when hollow-ground slides are not available is to place a drop of semen on a cover slip, which is inverted on a glass slide, each end of the cover slip being supported by other cover slips, thus providing space for the semen drop without pressure on the sperm (Lambert and McKenzie, 1940).

A warm stage should be used for the motility examination. This consists of a hollow metal block, placed on the stage of the microscope, through which flows water at a temperature of 38° to 40° C. Alternatively the microscope may be placed in a thermostatically controlled incubating chamber. If a warm stage or other means of preventing a fall in temperature is not used, a false low motility reading may be obtained. Care should be taken to avoid the use of cold cover slips and slides. Drying of the semen on the slide will also lessen motility. If the motility is poor on the first examination a fresh drop of semen should be examined after ensuring that the temperature conditions are optimum.

The various types of motility that can be observed are described elsewhere (p. 63). It is most important that sperm should possess progressive motion. Methods of estimation consist of some arbitrary scale for denoting the type of movement and the proportion of sperm exhibiting it. Thus, the relative number of sperm showing progressive movement may be expressed as a percentage, or in fifths or tenths of the total number of sperm. Gunn (1936), for example, assigned arbitrary numerical values to the various degrees of motility in ram semen and obtained a motility factor by multiplying the numerical value for the motility shown by the percentage of spermatozoa showing such motility, and dividing by 100. The estimation of motility is not an exact one but with experience an observer can form a fairly reliable opinion of the relative motility of different specimens of semen. The exact significance of different degrees of motility for fertility are not clear, but the picture of a first-class ejaculate should be impressed on the observer's mind and then deviations from this picture will at once be obvious. It is a comparatively simple matter to distinguish sperm with good motility and sperm with bad motility.

With a magnification of $\times 200$ to $\times 300$ it is possible to detect the movement of individual spermatozoa. The writer prefers to use a smaller magnification, with which one examines a larger field and estimates the motility of the sperm drop as a whole. The density of the sperm enters into this estimation, for the more active sperm there are present the greater motion they impart to the field under examination. In its highest form this motility is seen as characteristic turbulent wave or swirling movements. Slight activity takes the form of feeble forward or oscillatory movement. Ram sperm which is much denser than bull sperm typically shows the turbulent movement.

Enumeration of Spermatozoa

The number of spermatozoa is expressed per mm.^{3} or per ml. It is determined by means of a haemocytometer and red or white blood cell pipettes in exactly the same way as the enumeration of red and white blood cells. The same care must be observed in avoiding possible sources of error. Walton (1927) has described the errors of sampling. A 3% solution of sodium chloride is used for diluting and killing the spermatozoa. It is unnecessary to add a stain to the diluting fluid. Using a red cell pipette a 1 in 200 dilution is best for the semen of the ram, goat, and birds, and a 1 in 100 dilution for that of the bull; with the leucocyte pipette a 1 in 20 dilution is used for the semen of the boar, stallion, dog, and rabbit (Milovanov, 1934). This count gives the number of spermatozoa per mm.^{3}, which multiplied by 1000 gives the number per ml. The total number of spermatozoa per ejaculate can thus be calculated.

The bulk dilution method is considered by some workers superior to the blood-counting pipette (Walton, 1927 ; Belding, 1934). Day (1940*b*), for example, diluted 1 ml. of stallion sperm-serum with 99 ml. saline before making the haemocytometer estimation.

Rapid methods have also been developed. Burbank (1935) for the guinea-pig, Comstock and Green (1939) for the ram, Salisbury *et al.* (1943) for the bull, and Paršutin and Rumjanceva (1938) for the stallion, have used opacity methods, in which the concentrations of sperm in diluted semen samples are visually compared with opacity standards. A photometric method has been used for ram semen by Comstock and Green (1939) and for bull semen by Salisbury *et al.* (1943). The latter workers found that the concentration was determined by photoelectric colorimeter with an accuracy equal to that obtained with the haemocytometer. The visual comparison of diluted semen with opacity standards resulted in an estimate of concentration only slightly less accurate than that obtained with the other two methods. Directions are given for preparing opacity standards and for calibrating the standard tubes. Using the opacity method counts may be made on 4 ejaculates in 5 minutes or less.

Paršutin and Rumjanceva (1938) prepared a set of 7 test tubes containing sperm in concentrations of 10, 50, 100, 200, 300, 500, and 1000 million sperm per mm.^{3}. Visual comparison gives a near enough approximation as tested by sperm count.

Certain portions of the ejaculate may be low in sperm concentration, as in the boar (McKenzie, Miller, and Bauguess, 1938), and also in the stallion, jack, and dog in which the accessory secretions make up a large part of the ejaculate. Lambert and McKenzie (1940) therefore stress that care should be exercised to see that the sample is representative of the whole ejaculate. (*See also* p. 140.)

Hydrogen-ion Concentration

An electrical *p*H meter, using the glass electrode, provides a simple and accurate method of determining the *p*H. Colorimetric methods using suitable indicators can be used for approximate determinations. Webster (1939*b*) has prepared a colorimetric field testing outfit for stud ram breeders using B.D.H. Universal Indicator. Moore and Mayer (1941) used Nitrazine test papers for the rapid determination of *p*H ; they allow estimation to about 0·2 of a *p*H unit.

To determine the *p*H change, 0·5 ml. sperm is placed in a small Pyrex test tube and kept for one hour at a temperature of 37° C., the *p*H being determined before and after incubation (Anderson, 1944*a*).

Resistance

The resistance (R) of the lipoid capsule of sperm against the destructive action of 1% sodium chloride is expressed by the amount of that solution which must be added to sperm to arrest progressive motion (oscillatory motion may persist). It is determined as follows (Milovanov, 1934) : 0·1, 0·05 or 0·02 ml. of sperm is placed in a 200 ml. Erlenmeyer flask and 1% NaCl is added in small amounts, at first 10 to 20 ml. and then 2 to 5 ml. After each addition and thorough mixing the activity is rapidly evaluated under the microscope and in accordance with the results a greater or less amount of NaCl is again added. R is calculated from the formula $R = V/v$, where V is the volume of the solution and v the volume of the sperm, *e.g.* if the volume of the solution used for 0·05 ml. sperm was 78 mls. then $R = 78/0{\cdot}05 = 1560$. To avoid temperature shock the titration should be done at 15° to 25° C. and should not last longer than 15 minutes. To obtain comparable results, Nagornyĭ and Smirnov (1939) recommend the following technique : semen volume 0·02 ml. ; temperature 17° to 24° C. ; addition of NaCl in doses of 10 ml. with gentle stirring after each addition.

Morphology

Somewhat different classifications of abnormal or pathological types of spermatozoa are adopted by different workers (pp. 5, 13, 19). A considerable amount of practice is required in the recognition of abnormal forms.

The morphology is examined in stained smears, a large number of spermatozoa (333 to 1000) being examined, the size and shape noted, and the types classified. The number of abnormal types is usually expressed per 1000 spermatozoa.

Preparation of Slide.—A small drop of undiluted semen (semen may be diluted with physiological saline solution when the sperm concentration is high) is placed at one end of a glass slide. The edge of another slide is applied to the semen on the first slide which is lying flat on a firm smooth surface. The semen will now spread across the edge of the second slide, which, held at an angle of 45°, is pushed along the first slide, drawing the semen after it. McKenzie and Berliner (1937), however, state that

the best slides of ram semen were obtained by putting the flat surface of another slide over the slide carrying a fairly large drop of semen and carefully moving its whole surface over the slide without extra pressure. When slides were made by drawing the semen drop out with the edge of another slide the semen was often not spread in a sufficiently thin layer, or if too much pressure was applied the spermatozoa were damaged, for the tails break very easily.

Salisbury, Willett, and Seligman (1942) emphasised the need for a standard procedure for examining semen and the examination of more than one sample from each animal. They found that the most satisfactory method for the preparation of smears consisted of drawing a drop of diluted semen between two microscope slides, drying and clearing in chlorazene, and staining in carbol fuchsin and gentian violet.

Staining Methods :

Carbol-fuchsin-eosin.—For stock solution dissolve 10 parts of basic fuchsin in 100 parts 96% alcohol. For use add 10 parts of this solution to 100 parts 5% carbolic acid. Mix 50 ml. of this carbol fuchsin with 25 ml. of a saturated alcoholic solution of eosin. Keep at least 14 days before use. Filter on to sperm slides.

Williams' method after Lagerlöf (1934, 1936).—Allow the smear to dry in the air, and fix by heating just before staining. It is best not to stain for 2 to 3 days, otherwise the staining may be poor. Treat with 0·5% chloramine solution for one minute, or longer if necessary, to remove any mucus. Wash rapidly in distilled water, followed by 96% alcohol. Stain for 2 to 5 minutes with carbol-fuchsin-eosin (*see above*). Several slides are made and the correct time for staining found by trial. Wash rapidly in water. Counterstain for 2 to 5 seconds with Loeffler's methylene blue (30 parts of a saturated solution of methylene blue in alcohol+100 parts 0·01% aqueous KOH). Wash with water.

When properly stained the spermatozoa are usually very distinct and can be examined with an oil immersion lens. The spermatozoa assume a reddish-lilac tint, with the posterior part of the head darker than the anterior part. The tail becomes dark red in colour.

Carbol-fuchsin method (*McKenzie and Berliner*, 1937).—This method was used for ram sperm. Dry slides in air, then place in 10% solution of chlorazene for 5 minutes. Wash in distilled water and fix for one minute in a 10% solution of formalin. Wash again in distilled water and finally stain in Ziehl's carbol-fuchsin. Wash in running tap water and dry. This method differentiates the sperm head sufficiently without counterstaining with methylene blue.

Rose Bengal method (*Herman and Swanson*, 1941).—This has been used for bull semen. Dry the slide thoroughly and then stain for 5 minutes with a solution made as follows :—

Rose Bengal . . .	3 g.
40% formalin . . .	1 ml.
Distilled water . . .	99 ml.

After staining wash gently in distilled water and dry.

Method of Cary and Hotchkiss (1935) *modified by Gunn* et al. (1942).—This method has given uniformly satisfactory results with ram and bull sperm. It is an excellent stain for oil immersion examination. Fix with methyl alcohol for 2 minutes, wash in water and stain with Mayer's acid haemalum for 6 minutes. Wash in water for 3 minutes and then counterstain with an acidified saturated alcoholic solution of eosin for 2 minutes and again wash in water.

The head of the spermatozoon is stained blue, the attached end being more deeply stained than the front end, and the remainder deep red.

Mayer's acid haemalum is made up as follows :—

Haematin	1 g.
90% alcohol	50 ml.
Pot. Alum	50 g.
Glacial acetic acid . . .	20 ml.
Aq. dist	1000 ml.

Other Stains.—Stains which have been used for human spermatozoa include haematoxylin-eosin and aurantia (Lane-Roberts *et al.*, 1939) ; other methods described by Weisman (1941) are those of Cary and Hotchkiss (1934), Gelarie (1936), Holbert (1936), Williams *et al.* (1934), Williams (1937), and Pollák and Joël (1939).

Opal-Blue Method.—This method is used by Lagerlöf for determining the occurrence of drops of protoplasm on the necks of the spermatozoa (immature spermatozoa). A small quantity of an opal-blue solution (Opalblaulösung Bresslau) is heated in a test tube. After the solution has cooled, place 2 to 3 drops on a slide and mix with 1 to 2 drops of semen. From this mixture smears are made which are allowed to dry in the air. The spermatozoa appear as light negative pictures, with a dark blue background. The main shape of the spermatozoon is clearly defined and the delicate drop of protoplasm stands out clearly. (*See also* p. 140.)

Biometrical Examination

For the measurement of head length of spermatozoa a micro-projector is useful. Cheaper apparatus (Lane-Roberts *et al.*, 1939) consists of a microscope giving a magnification of 2000 times, to the eye-piece of which is fitted an inclined mirror, used for projecting the image on to a screen formed of a revolving plate, about 10 inches in diameter, covered with white graph paper (millimetre scale). The image is projected into the revolving screen in a dark room. Each spermatozoon is measured by turning the revolving screen so that one of the lines of the paper forms the axis of the spermatozoon whose head length can then be read off directly. The frequency of various head lengths is obtained and the coefficient of variation calculated.

A simpler method given by the same workers is as follows. A glass disc is fitted into the eye-piece (10 or 20×) of the microscope. Lines of various lengths are scratched with a diamond on this disc, and coloured with Indian ink or a grease pencil. They correspond in length to the sizes of the images of the spermatozoa. Celluloid can be used instead of glass. Spots or lines are made on the disc with Indian ink. By moving the slide with the mechanical stage the spermatozoa can be classified into groups, and the frequency distribution established with an accuracy sufficient for most purposes.

Metabolism

Glycolysis.—Comstock (1939) regards the measurement of glycolysis as a useful evaluation of ram sperm. In his method the semen is diluted with an equal volume of a diluent containing 16·6 g. Na_2HPO_4, 2·7 g. KH_2PO_4 and enough $CaSO_4$ for saturation in one litre of distilled water, and stored at 4° C. The original paper should be consulted for further details. Chemical methods, such as the Shaffer-Hartman-Somogyi, for the determination of glucose content may be used for studying glycolysis in semen.

Respiration Rate.—This was determined for bull sperm by Walton and Edwards (1938) using apparatus and technique as described by Dixon (1934). The bottles, about 35 ml. in volume, were of standard shape. The temperature of the bath was 37° C. and the rate of shaking 120 revs. per minute. The manometer differs from the standard Barcroft-Dixon type in respect to the side-tube; the modification enables determinations to be made without separate calibration of the bottles and is of considerable advantage when dealing with a large number of samples since all bottles are interchangeable. However, the results are slightly less accurate since alterations in the temperature of the room are reflected in the expansion or contraction of the air in the side-tube. But if the room temperature is kept fairly constant and the instruments are protected from direct radiation from gas fires, ovens, etc., the error is negligible.

For the determination of respiratory activity 1 ml. semen is mixed with 1 ml. of the following diluting fluid (Milovanov, 1934).

$Na_2HPO_4\ 12H_2O$	17·05 g.
KH_2PO_4	0·70 g.
$CaHPO_4$	0·05 g.
$MgHPO_4$	0·05 g.
Na_2SO_4	0·85 g.
Glass distilled water . . .	1 litre
Glucose	28·5 g.

The requisite amount of dry glucose is added to the solution immediately before use.

Manometer readings are taken every 10 minutes over a period of 2 hours and standardised to volumes of dry air at 0° C. and 760 mm. Hg. The curves obtained are plotted on squared paper and the initial and final rates obtained by drawing tangents to the curve at these points. These rates are further related to the number of spermatozoa and expressed as rates per million.

Comstock (1940) has described apparatus for field measurement of respiration of semen samples.

The principle is the same as that of the Haldane gas analysis and the Barcroft apparatus (modified by Dixon) for direct measurement of volume change. The equipment is portable and moderate in cost.

Examination for Abnormal Elements

Semen may contain bacteria, protozoa, or cells. Bacteria may occur normally in the semen of fertile bulls, but the presence of excessive numbers or of particular types may indicate a diseased condition. The cause should, if possible, be determined and doubtful males should be withheld from breeding until recovered from the abnormal condition.

Williams and Savage (1925) found primordial cells in only 3% of bulls, but their presence to the extent of 1% indicates a grave condition. The occurrence of epithelial cells, extruded cytoplasm, red blood corpuscles, and leucocytes was considered abnormal. Gunn and co-workers have described different types of germinal epithelial cells found in the ram.

Clinical Examination of Males

A clinical examination of the genital organs of males should always be made. In a model constitution for Livestock Breeding Associations, drawn up by the United States Department of Agriculture (1939*a*), the following health standards are required in the selection of each of the following species of sires :—

Bulls.—(1) Freedom from tuberculosis as shown by a negative tuberculin test made by an accredited veterinarian, (2) freedom from Bang's disease as shown by an official agglutination test,* (3) freedom from paratuberculosis as shown by the johnin test, (4) each sire to be subject to 90 days retests for above, (5) freedom from trichomoniasis as shown by clean herd history and negative clinical and microscopic findings, (6) freedom from other transmissible infectious and parasitic diseases as determined by clinical examination by an accredited veterinarian.

Stallions and Jacks.—(1) Freedom from glanders as shown by an official mallein or complement-fixation test, (2) freedom from dourine as shown by official complement-fixation test, (3) freedom from infectious abortion as shown by official serological tests, (4) freedom from other infectious diseases, such as coital exanthema, periodic ophthalmia, as shown by clinical examination by an accredited veterinarian.

Boars.—(1) Freedom from tuberculosis, Bang's disease, and swine erysipelas as shown by official tests, (2) freedom from other infectious diseases as determined by clinical examination by an accredited veterinarian, (4) immunisation against hog cholera.

Rams.—Freedom from infectious and parasitic diseases as determined by clinical examination by an accredited veterinarian.

These health standards are drawn up for American conditions. They would differ to some extent in other parts of the world according to local circumstances.

* Seit (1944) presents circumstantial evidence for the transmission of contagious abortion by a bull used in artificial insemination.

PART II

CHAPTER 8. ARTIFICIAL INSEMINATION OF ANIMALS

Advantages and Uses

The advantages and limitations of artificial insemination as a method of livestock breeding have been discussed by many writers, including in recent years Walton (1933), Hammond (1936), Miller (1938), Clapp (1938), Smith (1939), Cole (1939), Perry and Bartlett (1939), Lambert and McKenzie (1940), Hammond (1942), Anderson (1942*b*). There is little doubt that the method holds considerable promise for animal improvement. Waddington (1942) considered it the most important new biological technique at present passing into general practice. The very great progress made in Russia, and in Denmark and the U.S.A. where conditions in dairy herds are not greatly dissimilar from those in Britain, has focussed attention on the possibilities of artificial insemination in this last country. Technical progress has made its application a practical proposition ; the following are some of its advantages in comparison with natural mating.

1. *Increased use of sires.*—Artificial insemination provides a means of impregnating large numbers of females with sperm from one male, whose influence can thus be greatly extended. It allows the greatest possible advancement in dairy cattle breeding by making sires "proved" for milk and butterfat production available to a large group of herds, even distant herds, by the use of rail and air transport of semen. The best genetic stocks of cattle and sheep may be spread widely throughout the world at very small expense compared with the present system of conveying breeding animals by sea and rail (Hammond, 1942). [*See also* 9 *below.*]

2. *Evaluation of young sires.*—The reproductive life of sires is comparatively short ; one of the practical problems in animal breeding is to prove sires early enough in life so that the information may be used to discard the poor sires before they have done too much harm and to recognise and use the best sires as soon as possible. With artificial insemination young bulls might be proved at an earlier age than with natural mating ; progeny tests on bulls can be done on a more adequate scale and environmental influences offset to a certain extent by the use of such bulls on stock on different farms.

3. *Extending period of use of valuable sires.*—Because of age or crippled condition a male may be unable to serve properly or he may only be able to serve a limited number of females. By the use of artificial insemination the period of usefulness of such sires may be extended.

4. *Difficulties due to difference in size, etc.* may be overcome, such as the use of heavy bulls on yearling heifers. Crippled cows that cannot be bred naturally may be bred artificially (Clapp).

5. *Maintenance of satisfactory fertility.*—Artificial insemination can only be expected to give, at the best, results comparable with those from natural mating. However, certain parts of the procedure assist in obtaining and maintaining a satisfactory conception rate. These include (*a*) regular microscopic examination of the semen and the use only of high quality semen for insemination ; (*b*) keeping of good records and close attention to breeding performance ; (*c*) examination of cows for pregnancy ; (*d*) early detection of cases of sterility and their treatment. In the mare the percentage of conceptions might be increased by repeated insemination during heat. Insemination might be used to achieve this when service by the stallion is not available.

6. *Increased use of sires in monogamous species.*—In monogamous species artificial insemination offers a means of increasing use of valuable sires.

7. *In hybridisation.*—The method has been used in a few cases for making certain racial and species crosses, when natural mating has proved impracticable due to differences in size, anatomical structure or psychological characteristics.

8. *Aid in disease control.*—Artificial insemination provides a means of preventing exposure of sires to genital disease and thus indirectly of minimising the spread of disease. The indiscriminate use of bulls among several herds encourages the spread of genital disease and sterility. In herds of cows there are several important diseases which lend themselves to some measure of control by artificial insemination—contagious abortion, trichomoniasis, contagious vaginitis, so-called coital exanthema, while infection with genital tuberculosis and possibly with *Corynebacterium pyogenes* and other organisms might be added. One outstanding example of its successful use in this respect is in herds in Kenya affected with a specific form of contagious venereal disease (Anderson, 1939*a*, *b*, 1940,

1941). In a herd infected with genital disease it is possible, after the bull has been cured or replaced by a fertile, disease-free bull, to continue to fertilise females and to maintain or improve the birth rate without risk of spreading infection to healthy animals.

9. *Benefits to small herds.*—Smith (1939) held that the greatest immediate value of artificial insemination lies with its use in commercial herds, and particularly in small herds, where a distinct saving may be effected. The small man cannot afford to keep a bull for only a few cows, and if he does, he probably cannot afford to pay a large price for a bull. Nearly half the cows in England and Wales are probably included in herds with less than 20 cows. For this use of artificial insemination it will probably be necessary for a State organisation to take over the work, which is a logical extension of the " Scrub Bull Act." * Part of the large sums of money already spent on bull premium schemes might well be diverted to artificial insemination, and the quality of commercial cattle improved.

Similarly, Fowler (1942) and Hammond (1942) have stressed the limit to the breeding programme of the small farmer imposed by his pocket and the advantages which would accrue from artificial insemination in allowing him the use of well-bred sires at little cost. Hammond considers it of essential importance in dairy cattle, for the true genetic worth of a dairy bull is not known until his daughters come into milk, when he is at least 5 years old. Under present conditions the use of a bull of proved value is limited to one herd, or a small group of herds, where he will produce only about 30 or 40 calves a year. But with the aid of artificial insemination his progeny can be widely spread over the country, and by producing a thousand or more calves a year he will quickly and materially increase the efficiency of the dairy industry of the whole district in which he is kept.

Edwards (1941), in a survey of the county of Cambridge, showed that nearly $\frac{1}{4}$ of the dairy cows are in herds of 5 or less, and slightly more than $\frac{2}{3}$ in herds of 20 or less, while 1645 out of a total of 2160 herds have no herd sire. On the other hand, a relatively large number of bulls are in use in small herds (316 bulls in herds of 15 cows or less, some having more than 1 bull per herd) ; many are non-pedigree and even the purebred bulls are frequently of indifferent merit. The quality of the next generation is given little thought, yet more than half of the cattle bred come from herds of this size. Edwards pointed out that the cost of procuring a few first-class sires and of organising an artificial breeding scheme would be small compared with that of keeping the bulls required under the present system.

10. *General.*—It eliminates the need for taking females to the sire and *vice versa*. There is no need to keep a sire. Many animals can be artificially inseminated on the same day without the strain on the sire that natural service would entail. For cattle, the charge is no higher than yearly bull service cost per bull on most dairy farms.

Participation in a better breeding programme and study of mutual breeding problems brings forth the best thought and community spirit for advancement of breeding business (Perry and Bartlett).

Limitations and Objections

There is a certain amount of prejudice and misconception about the application of artificial insemination. Much criticism is ill-informed. Artificial insemination is carried out in accordance with thoroughly well-established physiological principles, and, in any attempts at making increased use of this method of breeding it is essential that full knowledge of the process be made available to all interested.

It is sometimes considered that artificial insemination will have some unspecified harmful effect on the progeny in future generations, but knowledge of the underlying principles of the method, together with its successful practice in several countries for many years, is sufficient to dispel any such doubts.

It is argued by some that the widespread application of artificial insemination may harm the present high quality of our breeds, studs and flocks. There is, however, states Fowler, no need for such a pessimistic view. This practice will allow the mass of breeders to take advantage of the benefits at present available to the few. As far as the skilled breeders are concerned, their art and skill in mating selected strains and families or in blending of strains will still be required to keep up the supply of bulls of high quality for stud purposes.

Another argument raised is that of the possible abuse to which the method lays itself open. Fowler does not consider this a serious stumbling block, for under existing conditions breeders are trusted in the stringent declarations about matings which they are required to make to their breed societies. Many breed societies have adopted rules whereby a stricter measure of control is exercised

* This work is now mainly to be undertaken by the Milk Marketing Board in England and Wales.—J. E. N.

on breeders for artificial insemination than for natural service, in that a competent authority must sign a certificate that he has carried out the operation. These rules provide a necessary safeguard and give assurance against the improper use of the method, with which matings in pedigree herds would perhaps be more accurately recorded and vouched for. As regards the country as a whole, some system is necessary to control the sale and use of semen of animals, for the method may get into disrepute and prove harmful if exploited commercially. In Britain regulations for this purpose have already been introduced.

Lambert and McKenzie (1940) emphasise that although artificial insemination is a promising procedure for the breeder, it can be used successfully and with safety only by skilled persons, such as specially trained veterinarians. A knowledge of the structure of the reproductive organs and of the technique for the collection of semen and insemination are necessary. Semen must be handled with great care if the sperm are to retain the full vitality necessary for the highest percentage of conceptions. Care must be taken also to avoid injury to the reproductive organs of both male and female and to prevent the spread of disease. As Fowler states, the fact that any co-operative scheme in artificial insemination necessitates constant supervision by a qualified person automatically provides a check against the onset of all reproductive maladies, an important factor in maintaining a high pregnancy rate and a proper schedule of breeding in farm stock.

Use in Colonies

The utilisation of artificial insemination in the Colonies may be considered from the viewpoint of European-owned and native-owned stock. The considerable experience of artificial insemination in European-owned stock in Kenya, which has already been referred to, may perhaps serve as a guide to other countries with similar problems and conditions. One further aspect of the application of this method of breeding in tropical and semi-tropical countries may be mentioned at this point. The importation of semen from temperate countries may prove most useful for tropical countries, where the difficulty of acclimatising a male of a European breed makes importation of sires expensive (Hammond, 1936). Under bad conditions in the tropics, European cattle may soon become degenerate and die, though crossbreds with local stock are more resistant to climate. It should also be possible for overseas breeders to obtain in other countries semen from particular sires whose blood they would otherwise be unable to incorporate in their herds.

There would seem to be certain basic breeding problems which are common, to a greater or less extent, to many countries. Fundamentally, the problem is one of genetic improvement of stock, making the fullest use of the best and most suitable sires that are available. There is a very large population of native-owned stock in British overseas possessions. Duckham (1932), for example, gave the total cattle population in the Colonial Empire as about 20 millions. What is the best method of improving such stock ?

Cattle.—It has been amply demonstrated in recent years that certain characteristics, such as capacity to withstand high temperatures (Rhoad, 1936), adaptability to local nutritional conditions and resistance to local diseases (Hammond, 1932) are developed to a much higher degree in indigenous than in exogenous cattle. Rhoad considered that the increased metabolism which ensues with temperatures above 23° C. is responsible for the low production records of European dairy cattle in the tropics. Edwards (1932) has shown that the combination of the productive capacity from the European breeds with the suitability to environment from the native breeds, results in an animal with greater production than either type under tropical conditions. Under the not too severe tropical conditions of Jamaica the proportion of about $\frac{3}{4}$ European to $\frac{1}{4}$ Zebu blood gives the best results, but under still more severe tropical conditions still more Zebu blood and less European might be required. The building up of new breeds of tropical cattle is now being attempted in this way in the Philippines (Manresa, 1939), in Jamaica and Trinidad (Hammond, 1932), the southern States of the U.S.A. (Black, Semple, and Lush, 1934), and in Queensland, Australia (Kelley, 1943).

The principles of genetic improvement of cattle in the tropics have been discussed by Smith (1931) ; he suggested that, as a general rule in tropical countries, the best improvement is obtained by selection within the native stock or by the importation of stocks which live and thrive in the tropics. The writer is of the opinion that this is the best plan for the improvement of Zebu stock in Kenya (Anderson, 1938*a*). Preliminary results, given by Anderson (1935), of the Ngong Veterinary Training Centre, Kenya, have indicated the milking potentialities of Zebu cattle, and they have been more fully demonstrated by later records, and also by records from other Veterinary Training Centres in Kenya. The great improvement in Indian breeds indicates that Zebu animals have very considerable productive resources well suited to their habitat. Breeding by selection within the Zebu

stock therefore holds considerable promise, and quicker results will no doubt be obtained by the use of improved bulls of Indian breeds, a few of which (Sahiwals) have already been imported into Kenya. Improved Zebu bulls from local stock farms should become increasingly available and with the importation of Indian bulls there should be a limited supply of better bulls for this breeding policy. Unless, however, the use of such bulls is combined with the practice of artificial insemination, it is difficult to see how it can, on the very restricted scale possible with natural mating, possibly effect any radical improvement in Zebu cattle (Anderson, 1939*a*).

This is the problem from the breeding angle, but it has to be fully considered from many other aspects, such as local circumstances and conditions, native customs and systems of agriculture, and so on. It is useless to consider the genetic improvement of stock unless environmental conditions are also improved ; unless grazing conditions and stock feeds are adequate ; unless drought, disease, and parasites can be adequately controlled. The difficulties of applying this modern method of breeding to stock, whose owners are relatively backward and uneducated, certainly cannot be underestimated. A breeding policy can therefore only be considered as part of a larger agricultural programme, which in turn is integrated with plans for the social development of native peoples. An increased milk production in native areas, together with increased local consumption of milk and milk products, would have far-reaching effects on the health and well-being of the native population in these areas.

It would seem that the part that artificial insemination might play in this development cannot but command the fullest attention once its possibilities are realised in responsible quarters. Its utilisation will demand energetic action on the part of the various Governments concerned. It will require adequate planning, guiding, and financing by the State. The most immediate problem is the investigation of the mating capacity of the Zebu bull, for the few trials that have so far been made with Kenya Zebus and Indian Sahiwals have shown them to be sexually lethargic. When it has been shown that semen can be readily collected from these and other indigenous types of bulls, centres should be set up in different districts and countries for the investigation of the problem on a practical basis. Such centres should be adequately staffed and equipped and should work in the fullest collaboration with those officers intimately concerned with native welfare.

Artificial Insemination of Cattle

There are three ways in which artificial insemination may be applied : (1) *Breeding within the herd.* This is done where the herd is sufficiently large to justify a self-contained insemination unit, or to prevent disease, or to make use of bulls incapable of natural service. (2) *Distribution of semen by the owner of the bull.* This plan is easy to develop, but may have many handicaps, according to Horwood, Cole, and Smiley (1940). The operator, in many cases, may not be properly trained in the technique of obtaining, preserving, and using semen. He may lack the qualifications for examining for pregnancy, and for handling sterility. Information on sanitation, and its importance in preventing the spread of disease is essential. Lack of these qualifications on the part of the operator may jeopardise the dairy industry of the community. In many instances the quality of the bulls may be inferior. Some national breed associations in U.S.A., it is stated, have already questioned this method of breeding from the standpoint of accuracy in registering animals. Many of these abuses may arise where the owner is distributing semen and is to profit directly by the volume of business. (3) *The operation of a co-operative breeding circuit.* This is the method of choice for the majority of farmers.

Artificial Insemination Associations

General recommendations concerning the organisation and the operation of dairy cattle breeding associations have been given by Herman and Ragsdale (1939) and Werner and Heizer (1940).

A pamphlet on the organisation of artificial insemination associations, based on the procedure drawn up by Professor Brownell of Cornell University, was published by the National Dairy Products Corporation in 1940. The New York State Extension Service has issued a fairly complete plan of work for promoting and organising artificial breeding circuits.

Organisation.—Associations are under the supervision of a board of managing directors or a managing committee elected from the members of the association. Inseminations are performed by a skilled operator, usually a veterinarian, who has had special training in the technique. The first step is a survey to determine the number of cows to be entered. Assurance of 1200 cows within a 20-mile radius is desirable for an artificial breeding co-operative.

Membership, Assessment, and Breeding Fees.—Income from membership and assessments is used for capital expenditure, and income from breeding fees for operating expenses. The usual

membership fee is $5. The suggested minimum for assessments is $1 for each subscribed cow. Assessments are usually levied at the time of joining the association to avoid the need for borrowing money outside the association membership. The assessment is generally considered an advance payment for the purchase of bulls, equipment, and other capital investment. As the sinking fund increases, assessments are returned to the members. The breeding fee is usually $5. This fee varies from $2 to $5.50 for 2 to 3 inseminations per cow; the New Hampshire Association charges $1.50 for additional inseminations and the Massachusetts Association makes a charge of $2 for a third insemination to the same animal. The Fleetwood Co-operative, Minnesota, charges a double service fee ($4.00) to non-patrons. A sample budget is given (National Dairy Products Corporation, 1940).

Headquarters.—The bulls and laboratory should be located near the geographical centre of the association's members' herds. A contract may be entered into for a farmer to supply the buildings, feed, and labour on a yearly basis; if this is done, precautions must be taken to prevent any possibility of the association's bulls becoming infected with any disease from the farmer's herd. Alternatively, property is leased or bought.

Management and Housing of Bulls.—Proper housing and management of bulls is of the utmost importance. Association bulls should not come into contact with other animals and should not be allowed natural service.

Bulls.—The bulls used in the organisation should be bulls of proven ability to transmit milk-producing capacity to daughters. Each bull should be tested before purchase for every health requirement. It is difficult to predict the transmitting ability of young sires and they should not be generally used throughout the circuit until proven. While one bull may ultimately be sufficient for 1200 cows, that is not possible at present. At least 3 bulls are desirable. If a second breed is added to the association, it may be possible to manage with one bull for the minority breed but in most cases two will be required. Arrangements should be made to raise and prove replacement bulls, so that a new group of sires can be procured every 2½ years to avoid inbreeding. In proving a young bull it is desirable to breed him to at least 50 cows and then retire him from breeding until the milk production of his daughters related to the production of their dams proves whether he is a good sire or not. (*See also* p. 140.)

General.—As a matter of policy, it is extremely desirable to inform each new member of the association, as he joins, that he must not regard his membership in the association as a panacea for all his breeding problems. It should be pointed out that artificial insemination does not necessarily make a " shy " breeder an easy breeder; that the rate of conception through artificial insemination is not materially better than by natural breeding, and will range from around 1·6 to 1·9 inseminations per pregnancy; that only 50% to 65% of all cows bred to artificial insemination will conceive on the first insemination; that a dam, unprofitable through her low milk production, cannot be made to drop high-producing offspring, even though she is mated, through artificial insemination, to the best of sires; and finally that his membership in an association does not remove the necessity and desirability of following sound culling and feeding practices. In other words, it is better to " under-sell " rather than " over-sell " new members on the advantages of artificial insemination in associations.

Rules and Regulations.—At the start of an artificial breeding association it is very desirable that rules and regulations governing the conduct of the association should be drawn up and approved by the association. Perry and Bartlett (1939) published rules and regulations typical of those governing the different artificial breeding groups in New Jersey. A Model Constitution and By-Laws for Livestock Associations have been drawn up by the United States Department of Agriculture (1939*a*). In Kenya a modified form of this Constitution, together with such other additional rules as were deemed necessary for the general management of the Association, has been adopted.

Use in Different Countries

The extent to which artificial insemination is used at present in different countries depends on a variety of diverse factors. In brief, it is used for three main reasons, (*a*) genetic, (*b*) economic, and (*c*) to avoid or overcome genital disease and sterility. In Russia, where very great progress has been made following the pioneer work of Ivanov (1907), in both the theoretical and practical aspects of this method of breeding, it is used for the first two reasons. In that country the shortage of sires, and the need for rapid improvement of livestock, were the chief factors which encouraged the start and growth of this practice, which has latterly been planned on an immense scale by the State. The aggregations of stock on collective farms undoubtedly aided the practical application of artificial insemination in the U.S.S.R. In other countries where the stock is not highly developed, where good sires are few,

and where farming units are comparatively large, insemination has been used to some extent. In Kenya the disease aspect has hitherto been the most important, but this phase is passing. In countries which have a highly developed system of agriculture, such as Great Britain and America, insemination has, perhaps naturally, been slower in gaining ground. In the early days of the practice of artificial insemination in these countries it was used in isolated instances, mainly on the larger farms. At the present day it is used to a considerable extent in organised breeding associations which provide for the insemination of cows on small farms. The highly organised and progressive farmers in Denmark were among the first, outside Russia, to apply artificial insemination on a communal basis. Denmark was followed by the United States, which has made rapid headway in the last few years. The position of artificial insemination in different countries has been reviewed by Bonadonna (1939*d*, 1941).

Scorgie (1943) stated that in England the true stimulus for the formation of artificial breeding associations has come from the breeders themselves. In Kenya, interest in this method of breeding on a communal scale has been voiced by the settlers themselves through the medium of local Farmers' Associations. Almost without exception the American associations have arisen as a result of a real demand for this service by the breeders.

Russia

In the U.S.S.R. in 1930 about 20,000 cows were artificially inseminated. In 1936 there were 1350 insemination centres for cattle with an average of 170 animals each. In that year 230,000 cows were inseminated (Keršin, 1937) compared with 165,000 in 1933 (Paršutin, 1934). According to Badirjan (1938) the 1938 plan provided for the insemination of 1½ million cows. There were to be 6282 insemination centres, each with a technician, and 1500 instructors. According to Neumann (1939) 1,200,000 cows were inseminated in 1938. By 1938 it has been stated that 50 million farm animals had been inseminated, and it is claimed that the use of artificial insemination has greatly speeded up livestock improvement.

In 1935 the average percentage of conceptions was 93·7, and in one district where 160 bulls were formerly required, 37 bulls impregnated 8000 cows. Keršin (1937) gave the maximum number of cows inseminated with sperm from one bull as 1090, and Neumann (1939) stated that one bull was used to inseminate 1536 cows, which produced 1490 calves.

Denmark

A co-operative breeding society of dairy farmers in Samsø started to use artificial insemination in 1936 (Sørenson, 1938). In 1939 there were 20 self-supporting societies for artificial insemination. By 1941 this number had apparently increased to 86 associations, with a total of 200,000 cows.

Italy

Bonadonna (1939*a*) has given a brief description of the work on artificial insemination in Italy leading to the establishment of the institutes for artificial insemination at Milan and Bologna in 1937. The former, the Lazzaro Spallanzani Institute, has now subsidiary institutes, and, by legislation, the Department of Public Health controls the organisation of local centres. About 100 of these centres were then stated to be working in Italy and her colonies, about 80% of their activities being with cattle. It is used chiefly to overcome sterility and any shortage of suitable males in certain areas, and also as a means of combating coital diseases. In 1938 and 1939 about 20,000 females were artificially inseminated. In cattle, up to 90% pregnancy has been obtained with one, two, or three inseminations.

United States

Artificial insemination has been used for several years in a few large herds of cattle and on large horse-breeding farms. Lambert and McKenzie (1940) considered it probable that such private use of the method will be confined to those herds that are large enough to justify the employment of a veterinarian or technician who can devote his services largely to this task. The greatest promise artificial insemination holds for livestock improvement in the United States is to spread the use of valuable sires. With improvements in technique in the last few years such an application is now feasible.

The first association organised to use artificial insemination in the United States was in New Jersey. By December 1938, 3 co-operative breeding units were in operation there (Perry and Bartlett, 1939). In 1940 there were 4000 to 5000 dairy farmers, with about 50,000 cows, enrolled in 54 associations located in 22 States. Trimberger in 1942 mentioned the existence of 64 associations. A central semen-producing unit called " The Central New York Artificial Breeders' Co-operative," made up

of 12 local inseminating associations or circuits, with about 6000 cows, started in June 1940 to supply semen to the local circuits. The Tri-County Breeders' Co-operative was in process of organisation in 1940 ; it contemplated breeding 5000 to 7000 cows, had a staff of 3 veterinarians (another to be employed soon), and planned to operate in 5 counties by sending semen from central bull quarters to veterinarians in the field. It was estimated that nearly 100,000 cows were artificially inseminated in 1941 (Rice, 1942). (*See also* p. 140.)

In an effort to improve the status of low-income farmers, the Farm Security Administration of the United States Department of Agriculture by July 1939 had organised more than 3500 breeding groups or associations having to do with dairy cattle and other forms of livestock. In a few of these associations artificial insemination was being used and steps were under way to use it in many more. Because of their limited resources these farmers could not afford to buy first-class sires, so the use of artificial insemination ought to prove of great value to this programme.

Great Britain

That Britain is lagging behind in the application of artificial insemination is greatly to be deplored, states Fowler (1942), since Britain has always been recognised as and called " the stud farm of the world." Hammond (1936) drew attention to the fact that too often in Great Britain the outstanding bulls are purchased and go to another country. Only those who can afford it can obtain the most desirable bulls. The Scrub Bull Act was put into operation to reduce the undesirable bulls, but this does nothing to increase the use of outstandingly good bulls. The *Farmers' Weekly* (February 19, 1943) stated that the proper market for top breeding stock should not be the Argentine or the Antipodes. Far more quality bulls than are available should be used in Britain. It was also stated that the disparity between the best of the pedigree breeders and the ordinary " commercial " producer is already far too wide and is growing wider.

A resolution passed by the Directors of the Highland and Agricultural Society of Scotland was to the effect that the Society objects entirely to the introduction of any scheme of artificial insemination for cattle in Scotland, as such a system is neither required nor desired in that country. The objections generally raised to this form of breeding have already been considered. It is doubtful if the fear that it may have harmful results on pedigree herds and flocks and may interfere with the sales of pedigree bulls is well founded. Experience in Denmark has shown that the use of artificial insemination has enhanced the price of bulls. It would seem not improbable that the existence of artificial insemination associations for cattle in Britain would increase the demand for high quality bulls. Whether the increased value of good bulls would affect yearling bulls is difficult to say (Smith, 1939) since only a few of the best would turn out as first-class proven sires ; but it might be stimulating in the same way as Argentine trade has stimulated the price paid for beef Shorthorns .

Scorgie (1943), in discussing these objections, states that one breed society has admitted that too many bull calves are being reared because of the possibility that they will fetch high prices ; he believes that artificial breeding schemes would do much to check the absurdly high prices now being paid for what are likely to prove, in many cases, inferior sires. Such schemes, moreover, would perhaps do much to eliminate the risk that after the war prices will drop and pedigree breeding suffer a set-back as bad as that after the last war. A further point mentioned is that the owner who is grading up his herd through artificial insemination will be provided with an incentive to acquire pedigree heifers, so that the trade for these would be more likely to improve than diminish.

With regard to the breed societies, Scorgie (1943) says that it is a common misconception that they are opposed to the practice of artificial insemination. The dairy breed societies have, in fact, drawn up regulations governing its use in pedigree cattle, and, provided these are adhered to, the Ayrshire, British Friesian, English Jersey, and Shorthorn Societies are prepared to accept the registration of calves born as a result of artificial insemination. The English Jersey Cattle Society have sponsored a scheme whereby the services of approved Jersey bulls can be made available to members, particularly those with small herds who might otherwise be compelled to use bulls of other breeds. The British Friesian Society are also considering what steps the Society can take in improving small herds by artificial insemination with semen from some of the best bulls of the breed.

Steps have been taken for the practical trial of artificial insemination of cattle, while ensuring adequate control by legislation of this method of breeding. Before its general adoption can be recommended, experience of its practical application under ordinary farming conditions is desirable. The Agricultural Improvement Council for England and Wales accordingly recommended trials of artificial insemination on a practical scale at two centres, the Cambridge School of Agriculture and the National Institute for Research in Dairying, at Reading, under the guidance of a Supervisory

Committee with the assistance of local committees for the two areas. The Cambridge and District Cattle Breeders' Society has accordingly been formed to operate the first non-profit co-operative breeding scheme, using Shorthorn and Friesian bulls. The breeding fee is £1 per cow for up to 3 inseminations, with an additional fee of one guinea to pedigree breeders in cases where an insemination certificate is required by the Breed Society. Control of the station and the field work will be in the hands of a veterinary surgeon employed by the Society. At Reading, Shorthorn and Guernsey bulls are to be kept, and arrangements are being made for use, on a limited scale, of a Friesian bull. Operations will be in the hands of a veterinary officer.

Post-War Development.—A report, prepared by the allied agricultural experts and considered by the Technical Advisory Committee on Agriculture (" Times," in Vet. Rec., 1943, 55, 76), revealed that the estimated decline of livestock in enemy occupied allied countries, as a result of lack of feeding stuffs, requisitioning and slaughter, was : cattle and sheep 11 million each, pigs 12 million, and horses 3 million. This decline constitutes a very serious menace both to post-war food supplies and to the future of European agriculture. Milk production has gone down by more than a third, and meat production by nearly half. Recovery to pre-war numbers of breeding animals will take many years.

A supply of good breeding animals in Europe after the war will be one of the greatest difficulties, and in re-establishment Russell (1942) considers that use will no doubt be made of artificial insemination. Waddington (1942) regards the method as of enormous potential importance for the immediate post-war reconstruction of the devastated areas of Europe. One of the first tasks, he states, will be the rapid increase of those animals which remain ; this should not be left to the control of the uneducated farmer or to the blind operation of economic forces. There will be, Waddington considers, an unparalleled opportunity for bringing about an important improvement in the general level of genetic quality in European stock. When the war ends, arrangements should have already been made for mobilising supplies of first-class male sperm and an organisation capable of shipping it to regions, both in England and on the Continent.

One of the most powerful arguments for the application of artificial insemination to cattle has been advanced recently by Kay (1942) in considering the future of the milk industry in Britain, which is the key to two matters of extraordinary national importance, namely, national nutrition and British farming. At present the average consumption of milk is just over 0·4 pint per head per day and the average quantity needed is not less than 0·8 pint, probably not less than 1 pint. On a yearly gallonage basis, the lower figure entails 1650 million gallons a year, *i.e.* about 140 million gallons a month, as consumption is evenly distributed over the whole year. This means a 75% increase in winter milk production and a 30% increase in summer production, assuming practically none for manufacture.

To meet these demands Kay considers that farmers would have to increase their efficiency and improve their management considerably. Various means of achieving this are discussed. From the 520 to 550 gallons of pre-war years, milk production could be rasied in a decade or so by the use of knowledge we already possess to 700 to 750 gallons. This could be done most rapidly, he considers, by taking every advantage of artificial insemination from first-class sires. More careful control of type of bull is advocated. The use of any bull inferior to the son of a proved sire out of a high yielding cow ought to be increasingly discouraged in any herd pretending to be a dairy herd. A simple system of milk recording would have to be made universal. Many of these requirements, and other related ones, could well be fostered and developed by artificial insemination associations.

Post-war planning of agriculture cannot but take full cognisance of the method. Present official livestock schemes are a step in the right direction but they are more of a negative than a positive policy. Livestock improvement is essentially a national problem, and if the advantages of breeding by artificial insemination are fully realised in preliminary practical trials, as they are likely to be, this method of breeding will call for adequate State planning and control.

Kenya

Artificial insemination in Kenya has been reviewed by Anderson (1939*a*, 1943). Artificial insemination was first used in Kenya in 1935 experimentally. By 1937 just under 6000 cows were artificially inseminated on 10 farms. At the end of 1941 the number of cows had increased to 15,000 and the farms to 36, on which 158 bulls (144 of them purebreds) were used for artificial insemination. On the basis of the 1936 cattle census on European-owned farms, the figures for 15,000 cows and 144 bulls represented 23% of the adult cow population and 26% of the purebred bull population. The adoption of artificial insemination of cattle on a communal basis was instituted in 2 districts in 1943.

The average number of cows inseminated per bull has varied from about 90 to 100 in each of

the 5 years since 1937. On the basis of the 1936 census, there was a ratio of 28·2 cows per bull for natural service. While there has thus been an increase of about three and a half times in the number of cows bred per bull, these figures show that there is room for a still further increase in this respect. The reasons why a larger number of cows have not been bred per bull include the retention for artificial insemination of the majority of bulls which had previously been used for ordinary service, this number being only gradually reduced, and the practice of some farms of running a number of bulls with the dry herd. Three farms, however, had above the average number of cows inseminated per bull, *e.g.* (1) 516 cows with 3 bulls, (2) 466 cows with 3 bulls, and (3) 258 cows with 2 bulls. On the other hand there were 3 farms with 50, 54, and 80 cows respectively, each using 2 bulls.

There was until recently little transport of semen. One farm of about 50 cows has for the last few years successfully used semen from another farm 20 miles away. On another farm with 366 cows, 3 bulls have been used for the home stock and a further 150 cows on a neighbour's farm, and it was intended to inseminate a further 200 head on a second neighbour's farm with the same 3 bulls.

In Kenya there are many herds numbering hundreds of grade cows, for which a large number of bulls was formerly required. Most of the larger farms have already adopted insemination and effected a considerable economy in the number of bulls, as well as increasing their effective service period. At present there are more bulls in use in these herds than are actually required, but it is necessary to remember that bulls (on account of illness, or other ill-defined causes) cannot be relied upon consistently to produce semen of good quality, and that it is essential to have a bull or two in reserve, as it is often difficult to replace bulls at short notice.

The main reason for the use of artificial insemination in cattle was the prevalence of genital disease (Anderson, 1941*b*), and there is little doubt that the prompt and widespread adoption of insemination has saved the dairy industry serious loss, and has assisted materially in maintaining production under present conditions. Of the 36 farms using artificial insemination at the end of 1941, 24 involving 11,910 cows had adopted insemination because of the existence of infection in these herds. Eleven farms, involving 3093 cows (20·6% of total) and 40 bulls (25·3% of total) adopted insemination for reasons of economy of bulls (5 farms also gave as an additional reason prevention of infection). Therefore, in only one-third of the farms, involving about one-third of the cows and one-quarter of the bulls, was artificial insemination adopted for reasons other than genital disease.

The limit to the use of the method has hitherto been on small rather than on large farms, but the better procedures now available for handling and storing semen have made the artificial insemination of cows on the smaller farms an eminently practicable proposition.

There is little doubt that considerable progress in cattle breeding in Kenya can be achieved by the adoption of artificial insemination on a communal basis, and a scheme based on experience with this method, together with available knowledge of its application on a communal basis in other countries, has recently been evolved (Anderson, 1942*b*). The first artificial insemination association in Kenya, the Trans Nzoia Community Bull Scheme, was formed in September 1941, but, largely because of difficulties in obtaining bulls, did not begin operations until April 1943. Guernsey bulls are at present being used and bulls of the Indian Sahiwal breed may later be used as well. The Limuru Cattle Breeders' Association was started in June 1943, Friesian and Ayrshire bulls being used. In both places committees have been elected to conduct the affairs of the Associations, and the technical operations are in the hands of Veterinary Officers. Full attention will be paid to reproductive disorders in members' herds, and these schemes should help materially in maintaining a satisfactory level of fertility in the two districts. It is not improbable that other similar associations will be formed in the next few years.

The main breeding practice with cattle in Kenya is to grade up by the use of purebred sires. There is little doubt that, so far, the genetic advantages of insemination have not been fully recognised, this aspect being still obscured by problems of genital disease. The use of artificial insemination in the present system of grading will assist greatly towards attaining uniformity of type and raising production. The choice of sires will have to be made more carefully, since their widespread use will have a greater effect for better or for worse. It will be particularly necessary to guard against breeding policy outstripping environmental conditions. The nutritional aspect is largely an economic one and can be overcome. Not so the climatic conditions, although almost the whole of the areas in Kenya where stock raising is already practised enjoy, as indicated by mean annual temperatures, a climate of the type regarded in other countries as eminently suited to the raising of cattle of the European breeds (Daubney, 1942). One or two districts are marginal in respect of atmospheric temperatures, and in them the progress of stock raising will have to be carefully watched, and where grade cattle are utilised those breeds which show the greatest tolerance to warm conditions should be selected.

Although artificial insemination is essentially a long-range breeding policy, its importance under war conditions in Kenya must be stressed. In this respect its value is based on two important factors, (1) the incidence of genital disease and (2) the practice of importing sires. There is little doubt that a reversal to natural service would cause a serious setback to the dairy industry on account of increased sterility in bulls and cows and a decrease in milk production. With natural service bulls would be exposed to the risk of genital infection and many would certainly become completely and permanently sterile. The difficulty at present experienced in importing bulls would thus be greatly aggravated.

There are also certain positive advantages which must be kept in mind. The present system of cattle breeding is dependent on the importation of purebred sires. The number of locally bred bulls is inadequate to meet the demand, and it is not at present possible to import the number of bulls required; the shortage will probably become greater as time goes on and bulls pass out of service. Artificial insemination should thus have an increasingly important part to play in making possible the fullest use of such bulls as are available.

The Committee of the East Africa Stud Book have drawn up regulations similar to those of certain breed societies in Britain for the registration of progeny born as the result of artificial insemination.

There is at present little attempt at controlled breeding of cattle by the African. Bulls of all ages and often of inferior quality are run with the herd and breeding is indiscriminate. The Veterinary Department has attempted to eliminate inferior bulls by castration, a policy which has met with some acceptance. Some improved Zebu bulls from Veterinary Training Centres have been issued to Bull Camps, where their services are available for African stock-owners. Their number, however, is so small that they cannot have more than a negligible influence on the stock as a whole. In some districts there is an appreciable number of African small-holders from whom there is an increasing demand for bulls. In one or two instances a small stud fee is charged by the African for the services of better bulls. A considerable number of Africans have a knowledge of artificial insemination, acquired from European-owned farms where it is practised, and its use for African-owned stock is favoured in some districts. There is little doubt that this method of breeding could be introduced into some of the more advanced communities with considerable benefit and with the approval of the African stock-owners.

Palestine

Bell (1938) has discussed the value of and the results from artificial insemination of livestock in Palestine, where the two main systems of agriculture at present are remarkable for their extreme diversity. On the one hand is the Arab peasant, whose agricultural practices have remained essentially unchanged for twelve centuries; on the other is the Jewish colonist, whose methods are the quintessence of modernism. The Jewish settlers are said to be developing this method of breeding with a view to organising its application as a routine practice in the breeding of dairy cattle and perhaps in the breeding of all classes of livestock.

Artificial insemination has proved to be effective in combating sterility and also in checking the spread of infectious vaginitis, and the preliminary results obtained interested the settlers in the economic advantages of the method. To test the practicability of artificial insemination 226 cows were each given a single insemination and 51·3% became pregnant; during the experimental period 172 cows were served naturally and 49·3% conceived. These results compare quite well with data from other countries; if 69 cows which were found to be suffering from diseases of the genital organs are eliminated the rate of pregnancy following artificial insemination rises to 68·6%.

From a veterinary aspect the method is likely to be of value in controlling the spread of dourine, contagious abortion and infectious vaginitis; Bell envisages a Palestine Stock-Breeding Service, which would have as its basis a country-wide network of artificial insemination stations. "Technical obstacles there are none," he states; "the difficulties are those of organisation."

Other Countries

The first organised artificial breeding society in Canada was formed in Waterloo County, Ontario, where a number of Jersey breeders have co-operated in the purchase of selectively bred bulls (McIntosh, 1942).

Artificial insemination is being considered in the Province of Orissa, India, as the province cannot afford to maintain an unlimited supply of good pedigree bulls (Solomon, 1941). There are 44 bull and 5 buffalo-bull breeding centres in the province.

In Ceylon a scale of fees has been drawn up for owners who wish their cattle to be artificially inseminated by the Veterinary Research Officer, Peradeniya (Crawford, 1941).

The bull-keeping association of Pinneberg (Holstein) adopted artificial insemination on a large scale in June 1942, the first to do so in Germany. Goerttler (1942) does not admit that the method is essential in German livestock breeding, as large numbers of good sires are available. Objections are raised to the method on several other grounds and it is considered that there is no necessity for its large-scale application in practice. According to Götze (1942) the need for adequately trained personnel and well-equipped insemination stations will necessarily limit its use at present, but the urgency of the matter is not great in view of the large stocks of good sires in Germany. In the denuded Eastern territories artificial insemination of cattle and sheep would be of invaluable assistance in increasing animal production rapidly, but where the essential technical conditions cannot be fulfilled, fertility will be much greater with natural mating. A special institute will have to be established in Germany, he states, to solve the many problems pertaining to reproductive disturbances, station equipment, techniques for collection, dilution, storage, transport of semen, and insemination, and to train veterinary surgeons and technical personnel.

Conception Rate and Factors influencing it

The very extensive application of artificial insemination to cattle by Russian workers has given most successful results. Some of the results which have been obtained in other countries are :—

Country.	No. of Cows.	% Calved.	Inseminations per Conception.	Authority.
U.S.A.	121	86·8	1·97	Cole and Winters, 1939
U.S.A.	517	97·3	1·80	Herman, 1939
U.S.A.	762	74·5	1·91	Henderson, 1939
U.S.A.	174	85·0	1·77	Kissileff, 1940
Denmark . . .	1070	87·6	1·68	Sørenson, 1938
Kenya	733	76·8	1·88	Anderson, 1938*a*, 1941*b*

When highly fertile bulls are used with normal healthy cows about 1·5 to 2·0 services are required per conception. The figures obtained from different countries for artificial insemination mostly fall within this range. Kingman (1936) used artificial insemination in a herd of 1500 animals, apparently with a ratio of about 2 inseminations per conception. Davis and Williams (1939) found a variation of from 1 to 4 inseminations per conception for different bulls with an average of 1·301. Burch (1939) inseminated 439 cows in a group of dairy herds with semen from 7 bulls ; the average of inseminations per conception was 1·99. Henderson's figures for individual bulls varied from 1·39 to 2·69 inseminations per conception with an average of 1·91. Anderson noted considerable variation between bulls used in one herd—a range of from 1·18 to 2·71 with an average of 1·79 inseminations per conception. Bartlett and Perry (1939) noted that young bulls generally have a higher conception rate.

On the basis of 1·5 to 2·0 inseminations per conception, from 50% to 67% of cows should conceive to a single insemination. Percentages of conceptions by the first and subsequent inseminations are :—

No. of Cows.	% Conceived to Insemination.				Authority.
	1st.	2nd.	3rd.	4th.	
1157	49·0	67·0	75·9	78·8	Sørenson, 1938
226	51·3	...	...	...	Bell, 1938
...	60-80	...	...	...	Bonadonna, 1939*a*
...	54·8	...	...	...	Hamilton and Symington, 1939
80	64·0	81·0	...	...	Hamilton, 1940
174	71·6	93·9	...	...	Kissileff, 1940
451	58·0	...	...	...	Anderson, 1941*b*

Burch obtained more pregnancies from the 1st insemination than from all subsequent. Henderson (1939) states that given relatively fertile animals, it would appear that approximately one-third must be expected to return after one insemination. Bartlett and Perry (1939), discussing the 5 co-operative artificial insemination units including about 5500 cows in New Jersey, state that for all inseminations the average rate of conception was 2·0. Trimberger (1942), on information from 64 artificial breeding associations in the U.S.A., found that any figure over 50% conceptions in females bred, or a requirement of less than 2 services per conception, could be considered a satisfactory breeding rate. (*See also* p. 140.)

Comparison with Natural Mating.—During one year artificial insemination was used on a herd of 61 cows at Ruakura, New Zealand (N.Z. J. Agric., 1942). In one half of the herd, inseminated under laboratory conditions, 62% conceived after one insemination and a further 14% after a second. In the other half inseminated under field conditions 41% conceived after one insemination and a further 25% after a second.

Winters and colleagues (1938) compared the service and insemination record in the Minnesota University herd, which was free from Bang's disease and had no other detectable disease that might have affected the breeding efficiency. The breeding record for the previous 5 years' service by the bull in this herd was 2 services per conception for 336 services. For 40 inseminations the number per conception was 1·25. Clapp (1938) states that conception from artificial insemination was almost equal to that from natural service. In the Michigan State College dairy herd, Horwood, Cole, and Smiley (1940) in the first year of artificial breeding found that 2·94 inseminations per conception were required compared with 2·92 in the previous year when natural service was used. In the main herd at Beltsville, 1·89 services were required per conception with natural mating; in the tuberculosis unit the cows were artificially inseminated and required 4·26 inseminations per conception (U.S. Dept. Agric., 1939*b*). This unusually high ratio was due primarily to the low fertility of the semen from 2 of the bulls used. In the Naivasha Experimental Station herd, previous to the use of artificial insemination, the number of natural services required per conception was 1·59 (433 services; author); in this herd the percentage of cows calving annually when artificial insemination was the sole method of breeding varied from 78·6% to 91·5% over a 4-year period, while in the previous 8 years 80% fertility was obtained from natural service.

Shuart and colleagues (1942) compared natural and artificial mating in heifers bred to the same sire. There was a significant difference between the two groups; the 22 animals artificially inseminated required 1·06 inseminations per conception, with 90·6% conceiving on 1st service, while the 22 heifers bred naturally required 1·67 services per conception, with 63·6% conceiving on 1st service. Berousek (1942) states that a survey of the literature concerning the artificial breeding of over 10,000 dairy cows indicates that approximately 1·7 inseminations per conception were required as compared with 2·2 services per conception for approximately the same number of cows in various herds where natural breeding was practised. To compare with artificial breeding efficiency, half the Missouri Station dairy herd was bred naturally and the other half artificially for a 2-year period. During this period artificial breeding required 1·59 inseminations and natural breeding 1·66 services per conception. He gives the following data :—

Breed.	No. of Bulls.	Natural Mating Inseminations per Conception.			Artificial Mating Inseminations per Conception.		
		No. of Conceptions.	Mean.	Range.	No. of Conceptions.	Mean.	Range.
Holstein . .	8	209	1·66	1·32-2·02	127	1·89	1·00-5·00
Jersey . .	5	151	1·59	1·38-4·66	106	2·16	1·32-4·25
Guernsey . .	1	49	1·58	...	20	1·67	...
Av.	...	...	1·63	...	...	1·98	...

There were wide variations in individual service rate. Four bulls had a lower rate when used artificially than when used naturally. The lower efficiency for artificial breeding was not considered

significant because in many cases stored semen was used. All factors considered, it was concluded that there is little difference in the results of natural and artificial breeding.

Miller and Graves (1932) found that the percentage of females bred that conceived (natural breeding) in a herd of registered Friesians was 86·3%, and the percentage calving to artificial insemination is very similar. Of cows that calved to artificial insemination over 90% did so in up to 3 inseminations (Davis, Kissileff, Anderson).

Insemination in Different Herds.—It is only to be expected that insemination will give different results in different herds. Winters and colleagues (1938) instanced 3 herds which had ratios of 1·25, 1·5, and 4·20 inseminations per conception. The second herd was only recently started and had no previous records. The third herd had a poor breeding record. Insemination was practised on only half the cows, and they were those which the owner was experiencing most difficulty in getting settled. This herd illustrates the conditions under which insemination is frequently used and in which good results cannot be expected. Herman (1939) states that breeding efficiency, even where no genital disease occurred, varied considerably among some 50 Missouri dairy herds. In 3 herds in Kenya in which artificial insemination was used (Anderson, 1941*b*), 1·72 (Experimental Station), 1·76 and 2·53 inseminations per conception were required. The 2 latter herds, and particularly the last, were affected by the contagious venereal disease peculiar to Kenya which is associated with vaginitis in the cow and epididymitis in the bull (Daubney *et al.*, 1938 ; Anderson, 1939*b*, 1940). The prevalence of this disease was probably responsible for the lower conception rate. The Experimental Station herd has never been affected by venereal disease and has been free from contagious abortion since 1935.

Cole (1938) inseminated cows in 7 different herds with semen from one bull. One herd showed a low breeding efficiency both from artificial and from natural insemination in previous years. Another herd, free from disease, showed much better results from artificial insemination than from ordinary service in the preceding 5 years. Bartlett and Perry (1939), discussing the 5 co-operative units for artificial insemination in New Jersey which included about 5500 cows, said that in some herds inexplicably poor conception rates occurred ; some bulls are apparently incompatible with certain cows.

Time of Insemination.—The optimum time depends on the relationship between the time of ovulation and the effective period of survival of sperm in the female genital tract. Hammond (1927) found that ovulation occurred 24 to 48 hours after the beginning of heat. Werner, Casida, and Rupel (1938) found that in the great majority of cases, ovulation occurred before the 3rd half-day following the end of heat. These data support the opinion that the optimum time for introduction of sperm is late in the heat period or soon after its close. The failure of a comparatively large number of cows to conceive after a single insemination or service may be due to a too lengthy interval between the time of introduction of sperm and ovulation, as for example, by introduction of sperm too early in heat.

Paršutin and Verevkina (1932) found that the most favourable time for insemination was 18 hours after the beginning of heat. Herman (1939) found that among 1139 cows fewer inseminations per conception were required when breeding occurred within 12 hours of the first signs of heat. Hamilton and Symington (1939) obtained greater success in settling cows in the later stages of œstrus. Using a rough classification of the stage of heat, Henderson (1939) obtained best results in middle and late heat. Burch (1939) found that cows inseminated from the 18th to the 24th hour after the onset of heat gave the best results ; services earlier or later than this were less satisfactory. Bartlett and Perry (1939) observed that conception was highest during full (8-12 hours) and late (12-24 hours) oestrus. Horwood, Cole, and Smiley (1940) found about 49% of conceptions on the day of heat compared with about 26% on the day following heat. Trimberger (1942) states that experiments at Nebraska have indicated that it is best to breed in the middle or towards the end of heat, although good results are obtained as late as 6 hours after the end of oestrus ; beyond this point poor results were obtained. (*See also* p. 141.)

Two inseminations during a single heat period have given much better results than one, only 10% of cows coming on heat again (Andreev, 1937) ; this procedure was also recommended by Kirillov (1937). This observation, however, does not apply to grade cows in Kenya, due perhaps to the relatively short period of heat experienced by these cows (Anderson, 1941*b*). Female reproductive functions are susceptible to the influence of various factors such as breed, individuality, season, climate, which must be kept in mind when considering the question of the optimum time for insemination.

Age of Cows.—Commonly, in Kenya, heifers are rather more difficult to get in calf. Werner, Casida, and Rupel (1938) observed that heifers required significantly more services per conception than cows.

Most of the difficulty with heifers occurred where an old bull was used, but when this same bull was used on cows his service record was satisfactory. Bartlett and Perry observed that young bulls seemed to settle 1st calf heifers at a higher rate than mature bulls. Dunlop (1941) advised against the use of old bulls on young heifers; in the majority of cases it is not successful, although the reason is unknown. Burch (1939) found that virgin heifers conceived more readily than mature cows, probably owing to lower incidence of disease. (*See also* p. 141.)

Site of Injection.—Siebenga (1938) reported the following percentages of pregnancies from the introduction of sperm into different parts of the genital tract: cervical insemination with undiluted semen 90% (199 inseminations); cervical insemination when part of the semen flowed back into the vagina 88% (33 cases); uterine insemination 40% (10 cases); vaginal insemination 11% (9 cases). (*See also* p. 141.)

Range Cattle.—Lasley, Montgomery, and McKenzie (1940) have reported the results of artificial insemination in range cattle. Of 263 registered Hereford cows artificially bred in 1939, 79·8% calved; 323 inseminations were required, an average of 1·54 per calf. Between April and August 1940, 725 cows were artificially bred, requiring 954 inseminations. With natural mating a calf crop of only 40% to 65% is secured.

Gelatinised Semen.—Maruškin and Sivokonj (1939) used cervical insemination with gelatine capsules; in tests comprising 142 cows the percentage of calving was 89·4%, which differed but little from 94·5% obtained by the usual method of insemination. Davis, Underbjerg, and Trimberger (1940), using a 2 cm. cylindrical gelatine capsule, deposited semen in the cow's vagina by means of a metal inseminating gun 21 inches long with a movable plunger. Only slightly less efficient conception results (1·63 inseminations per conception) were obtained than with cervical inseminations (1·46).

Artificial Insemination of Sheep

Application in Different Countries

Russia

Ožin and Paršutin (1934) state that artificial insemination has been widely used in sheep breeding in the U.S.S.R. since 1930. In 1930, 100,000 ewes were inseminated, in 1931, 600,000, in 1932, 1,600,000 and in 1933, 1,630,000. The percentage of pregnancies obtained was high. Thus, out of 920,000 ewes inseminated in 1932 on the State sheep breeding farms, 82% became pregnant, compared with 80% in 1,560,000 ewes served naturally. In one district the average number of ewes inseminated from one ram was 132. The authors recommend a more thorough study of the rams by the progeny test and the more intensive use of the best sires.

Kulešenko (1937) gave an account of artificial insemination of sheep in the Orlov district of the Azov-Black Sea Region, which has a population of over 70,000 sheep, mainly Merinos and their crosses. The Rambouillet Merino is used for grading up. In 1936 the total ewe population was inseminated artificially with the sperm of 8 rams, the average number of ewes per ram being over 5000. One ram performed 372 matings and yielded 557 ml. of semen, which was used to inseminate over 15,000 ewes. Altogether 13 inseminating centres were operating and the greatest distance over which semen was transported was 300 km.

Keršin (1937) reported the progress made in the various districts of the U.S.S.R. during 1936, when a total of 6,450,000 ewes were inseminated, which was 3 times more than in 1935. Eight thousand insemination centres were in operation with an average of 800 animals. The average percentage of conceptions in 1935 was 97.

In 1933 one Rambouillet ram sired 2580 lambs, and the best Karakul ram sired 700 lambs (Paršutin, 1934).

Ožin (1939) states that in 1938, 14,500,000 ewes were artificially inseminated, involving the use of 41,274 rams, with an average of 280 ewes per ram; nearly 250,000 more rams would have been required for natural mating.

Kenya

In the Experimental Station flock a total of 2540 ewes were inseminated in the period 1935 to 1941, and the average birth rate was 64%. In different years the lambing varied from 47% to 79%. This figure is somewhat lower than it might have been because of certain experimental procedures and lack of knowledge of the optimum season for breeding. From natural service a 75% lambing is considered satisfactory in Merino sheep in Kenya, but there is considerable variation from year to year (Anderson, 1937, 1939*a*, 1941*c*, 1943).

The method has been used by the farmer himself on 4 large sheep farms. In the seasons 1936-37

and 1937-38, 716 and 1400 ewes respectively were inseminated and 76% and 65% of the ewes lambed. On two farms (G and S) under the same management, using artificial insemination, the total figures for 1938, 1939, 1940, and 1941 were : ewes 1340, 3669, 3042, and 2875, and birth rate 62%, 76%, 68%, and 72% respectively. Insemination was carried out for about 7 weeks, after which rams were run with the ewes. The figures for the combined practice of artificial insemination and natural service were 66%, 86%, 70%, and 75%. In 1941, on another farm, 490 ewes were artificially inseminated, 80% lambed, producing 92% lambs. The total number of ewes inseminated by the farmer himself has thus increased from 1340 in 1938 to 3375 in 1941, and the average lambing was 76% for this period.

In the Experimental Station flock of high-grade Merinos no attempt has been made to inseminate the maximum number of ewes with one ram, but the number of rams actually used was considerably less than when ordinary service was practised. In this flock of 600 ewes an average of 3 rams was used per breeding season in the period 1935-1939. In 1940 and 1941 the same ram sufficed for insemination of about 500 ewes in each year. In the period 1935-1939 an average of 348 ewes were inseminated by one ram and an average of 191 lambs were born to one ram. In 1937 one ram sired 411 lambs. On one farm in 1938 one purebred Australian ram was used to inseminate 1340 ewes, of which 887 (62%) lambed. On farms G and S 3 rams have latterly been kept for 1500 ewes, but 2 have usually sufficed.

In the larger European-owned flocks it is usually impossible to provide an adequate number of purebred rams ; grade rams have to be used to some extent. Since all purebred rams used are imported, the use of artificial insemination should therefore be especially valuable in wartime.

In sheep there is a rather better realisation of the genetic possibilities of artificial insemination than in cattle. The resulting economy in rams has permitted the greater use of such better rams as are available. However, the selection of rams is entirely phenotypic and it is very necessary that the choice should be placed on a genotypic basis by the institution of a practical method of progeny testing of rams.

As regards native sheep there is considerable scope for the use of rams of mutton and wool types in those parts of Kenya suited to the particular types of sheep. The possibilities of the more widespread application of artificial insemination to sheep in Kenya are indicated by the results which have been obtained.

Romania

Teodoreanu (1939) reported that in experiments on artificial insemination of sheep, 96% fertility was obtained with a dose of 0·1 ml. The percentage of twinning was similar to that from hand service, and the birth weights and vigour of the lambs (male) were the same as with natural mating.

Cardaş, Paşcovschi, and Nica (1939) state that the arid plains of Bessarabia represent the most important Karakul breeding area in Europe, apart from Russia. The industry has been greatly hampered by lack of good stud stock. The possibility of artificial insemination was first considered in 1936, and by 1938 13 stations were set up in several districts. The 8122 ewes inseminated required 15,054 inseminations, about 29% coming on heat a second time, and 9% a third time. The average fertility rate of 91% for all stations was considered satisfactory.

South Africa

Quinlan, Maré, and Claassens (1936) found that single insemination into the cervix at one heat period (81 Merino ewes) gave results (of from 33% to 56%) almost as satisfactory as hand service (57%), and at least 4 times as many females could be impregnated from the same number of ejaculations. They consider that, in a country like South Africa, if the operations were universally adopted the stud breeder would suffer, as less males would be sold annually. It is possible to replace rams by as good or better sires from a stud breeder. They state that the technique of artificial insemination is so complicated that it is unlikely to be of economic value to the rural population of South Africa in the normal breeding of domestic animals, but more intensive use may be made of exceptional sires in large stud flocks and the services of an experienced technician will then be warranted. (*See also* p. 141.)

Australia

Gunn (1936) inseminated 136 ewes under somewhat unfavourable conditions, and 13 lambed. Kelley (1937) obtained 22% of lambs from artificial insemination of 55 ewes. A large-scale trial in one of Australia's Merino studs has recently been made (Kelley, Granger, and Gunn, 1942). The original publication must be consulted for details. Kelley summed up his genetical discussion: " Artificial insemination thus has advantages and disadvantages. It is a process well adapted to supplement hand service in studs if the requisite technical skill is available. It is a means and not

an end in itself, and is properly associated most intimately with adequate genotypic selection. As such it ranks equally with inbreeding, selective mating, whether of likes or unlikes, and the other tools of trade with which the animal breeder works. It certainly is not a process to be implemented lightly or without due consideration." Granger found that the method was practicable under good hand-mating conditions, when performed by, or under direction from, a skilled person. The proportion of ewes which conceived was comparable to that from hand serving.

Gunn considered that in Merino flocks on station properties and even in fat lamb raising on farms, the main objection to the application of artificial insemination will be that of cost, which under present conditions is likely to be prohibitive. The quality of station sheep could, however, be quickly improved by the use of proven sires. With stud sheep the relatively high cost of artificial insemination is not a bar to its adoption. Gunn stressed the value of the process in progeny testing of young rams and in the wider use of proven ones : " Progeny testing might even be commenced on rams as young as 6 to 8 months ; hence the ram's value might be known to an extent by the time he was 2 years old, and known thoroughly by the time he was 3 or 4 years old. Then, by extending the use of a proven ram many-fold by artificial insemination over the remainder of his life his ' blood ' could be disseminated throughout the top stud sheep to whatever extent was found wise and a tremendous effect produced on the quality of sale rams from that stud."

Argentina

García-Mata and Cano (1941) described the technique of collection and insemination used, whereby 120 sheep can be inseminated per hour (method used on 6300 sheep). Ross (1942) has discussed results of artificial insemination of sheep in the province of Buenos Aires. In the 1st season 108 Lincoln ewes were inseminated with sperm from a proved Lincoln ram which was incapable of natural service owing to an injured back. 52 were impregnated at the 1st insemination, 29 at the second, 21 at the 3rd, and 6 not at all. 8 ewes could be inseminated with one ejaculate. In the following year 172 Lincoln ewes produced 219 lambs by artificial insemination, and 300 Corriedales just over 300 lambs. In 1941, 6500 Lincoln and Romney ewes were inseminated with sperm from 9 rams. The inseminations were performed at 3 stations where revolving platforms containing 3 stalls enabled 110 ewes to be inseminated per hour by 2 operators.

Factors affecting Insemination Results

Season.—Merino ewes are capable of experiencing a continuous series of dioestrous cycles throughout the year when conditions are favourable. In South Africa it has been observed that Merino ewes, under Western Free State conditions, have a prolonged anoestrous period (Kupfer, 1928 ; Roux, 1936), and in Australia, Kelley and Shaw (1939) have shown that Merino ewes show a well-defined periodicity in the percentage coming on heat ; there was a fall in the incidence of oestrus in the spring months, followed by a rise in the summer months, the higher levels being maintained during late summer and autumn. In Kenya there is a marked seasonal variation in reproductive capacity in Merino ewes. In general, it may be said that it is in the early months of the year that the incidence of oestrus is highest and the cycle shortest. Therefore the best results should be obtained from breeding operations at this season of the year. The results obtained from artificial insemination support this view.

Even during a restricted breeding season changes occur in the reproductive activity (Grant, 1934 ; McKenzie and Terrill, 1937 ; Cole and Miller, 1935) and in fertility (Marshall, 1905 ; Roberts, 1921 ; Nichols, 1927). It is thus apparent that full attention must be paid to seasonal variations in reproductive activity in both ewes and rams, if the best results are to be attained from artificial insemination.

Time of Insemination.—The optimum time for insemination depends mainly on (1) vitality of sperm in the genital tract of the ewe (*see* p. 82) and (2) the vitality of the ovum. The concensus of opinion is that the life of the ovum is very short in Merino and other ewes (Quinlan *et al.*, 1932 ; Anderson, 1941*c*). Ovulation takes place as a rule about the end of oestrus (McKenzie and Terrill, 1937 ; Anderson, 1938*b*). In Merino ewes Kelley (1937) found that ovulation took place after the end of oestrus. In grade Merino ewes in Kenya, ovulation also occurs shortly after the end of oestrus, but there is a minimum period of about 23 to 25 hours after the onset of oestrus, before which it was not observed to occur even if the duration of oestrus was much less than this interval.

There should be available at the time of ovulation a sufficiency of sperm of high vitality, and this could probably be achieved by the introduction of sperm at, say, 5 to 6 hours before the time of ovulation (Kelley, 1937 ; Anderson, 1941*c*). The effective life of sperm in the ewe is, however,

probably considerably longer than this, and the introduction of sperm earlier than 5 to 6 hours before the time of ovulation probably gives good results. Warbritton *et al.* (1937) stated that of 3 periods of insemination tried, 12 hours before ovulation was the most desirable ; for many ewes this meant breeding 10 to 18 hours after the onset of oestrus. Kardymovič *et al.* (1934) found that the optimum time was 18 to 25 hours after the onset of oestrus (duration of oestrus not stated) ; at this time the percentage of conceptions was 85, but at 26 to 42 hours after the percentage had fallen to 48, presumably because of a number of ewes having gone off heat before being inseminated. Zajac (1935) found that the optimum time was 24 hours from the beginning of heat, but even at 48 hours 77% of the ewes conceived. Since the time of ovulation is usually related to the end of heat, the optimum time for insemination depends on the duration of oestrus and this will vary in different breeds and under different conditions. Kelley (1937) for example, obtained a higher percentage of conceptions with matings within a maximum of 4 hours from the onset of oestrus in Merino ewes than in Dorset ewes, which had a longer oestrous period.

Number of Inseminations.—When rams are run with the ewes, each ewe is probably served several times at intervals during one heat period. However, a single service has been found to give similar results to two or more services during the one heat period, although Kelley (1937) found a difference in favour of several services. On the whole, in artificial insemination experiments on high-grade Merino ewes in Kenya, somewhat better results have been obtained by giving 2 or more inseminations per heat period, but the inseminations were not always carried out at the same season of the year and the results are not therefore strictly comparable. From experiments on 1100 ewes Kirillov (1938) concluded that all ewes should be tested for heat twice a day, morning and evening, and only those with a heat period of more than 24 hours should be inseminated twice (the duration of heat was under 24 hours in 47% of these ewes and 24 to 36 hours in 45% ; 3 or 4 inseminations did not raise the rate of lambing of ewes with heat periods lasting over 1½ to 2 days. Avramov (1937) inseminated 106 ewes twice at an interval of 8-12 hours or 20-24 hours. When the interval between inseminations was 24 hours, the lambing rate of Ţigai ewes was raised by 11·7% and of Chushka by 14%. Gavrilov (1937) found that an 8-hour interval gave somewhat better results than the 24-hour interval and suggested that the 2nd insemination should be carried out not later than 12 hours after the 1st. The principal cause of the increase was the larger number of twin births, though barrenness was also reduced.

According to Milovanov (1934) the results of repeated insemination have been excellent in some cases and negative in others. It was stated that it will be effective if the ewe is genetically capable of producing more than one ovum, if environmental conditions are favourable, if the interval between ovulations is long, and if the sperm is of low vitality. The same author (1936) reported that some Russian sheep stations experienced a large increase in lambing percentages when the ewes were inseminated several times. Peregon (1936) inseminated 56 Karakul ewes twice at an interval of 20-30 hours ; they produced 96 lambs (171%) whereas the average for the flock was 115%. Lopyrin and Loginova (1939) made observations on multiple insemination of 2582 ewes, and on the time of ovulation (average of 30-32 hours after onset of heat) and duration of survival of sperm in the female genital tract (average 32 hours). Little difference was noted between ewes inseminated once and ewes given 2 or 3 inseminations at 16-hour intervals.

Gelatinised Semen.—Milovanov (1938) investigated the use of gelatinised semen for insemination of sheep. Semen was diluted $\times 3$ to $\times 13$ times—diluent GPS-2-G being more satisfactory than GPS-7-G—and poured into reed capsules 150 mm. $\times$ 6-8 mm. Each capsule contained 1 ml. semen or 500×10^6 sperm. The capsules were packed in cotton-wool and waxed paper and sent by aeroplane to the farms where they were received after 20 minutes to 6 hours. Each capsule was introduced into the vagina of the female where the gelatinised semen was pushed out by a wooden or glass rod, after which the empty paper capsule was removed. 1254 ewes were inseminated and 1228 lambs were obtained. The average rate of fertilisation after a single insemination was 74%. Observations on control capsules (which were dropped from an aeroplane) revealed that all those opened after an interval of 8 to 10 hours contained living and active sperm ; after 30-36 hours activity was reduced, and after 40-48 hours the sperm were usually dead. Razumov (1938) placed gelatinised semen in paper capsules, which were transported in a thermos flask at 15° C. The interval between collection and insemination was not stated. Forty-seven ewes were inseminated twice during one heat period and 43 ewes are said to have produced 102 lambs.

Smirnov-Ugrjumov and Gordeeva (1939) used gelatin capsules, paper capsules, and capsules made of cocoa butter for cervical insemination in experiments on 300 ewes. With gelatin and paper capsules, doses of 0·3 ml. [dilution and number of sperm not stated] gave as good results as insemination with the syringe (0·10 ml. into cervix), whereas doses of 0·10-0·15 ml. into the inner portion of

the vagina without a speculum were inadequate. Nagornyĭ and Smirnov (1940) compared ordinary medicinal capsules of 0·5 ml. capacity, treated with melted paraffin and containing 0·2 and 0·4 ml. semen, and capsules treated with "nitrolac" containing 0·2 ml. The total lambing was 71%, which was practically the same as from natural mating. The dose of 0·4 ml. (into the vagina) gave the best results. They consider that the great simplicity of this method renders it very valuable, especially in small flocks. The dose recommended is 0·3 to 0·4 ml., and though this is rather high, cervical insemination is not advised as being too complicated.

Artificial Insemination of Horses

According to Paršutin (1934) 40,000 mares were artificially inseminated in the U.S.S.R. in 1933, and in 1937 the number is stated to have been 50,000. Paršutin and Paryšev (1938) state that the plan for 1939 provided for the insemination of 276,550 mares at 1871 stations.

Životkov (1933-34) reported that 376 mares were artificially inseminated and 260 (69·7%) conceived, which was almost exactly the same result as that from a combination of artificial and normal mating. Among 25 mares inseminated with diluted semen, the percentage of fertilisation was highest with a 1 : 1 dilution. Failures were most frequently due to inadequate testing of mares. Životkov (1935) considers that artificial insemination of mares would yield much better results if attention were paid to the following : exact diagnosis of oestrus ; dosage of sperm injected ; a study of the pathology of the genital organs of the mare. Patrušev (1935) found that only fairly large doses—20 ml. or more of undiluted sperm—gave satisfactory results. Diluted semen, even in larger volumes, was unsatisfactory. Salzmann (1937) obtained good results with insemination into the uterine horns.

Životkov, Krakov, and Antonenko (1939) reported experience gained at an army stud of 322 mares. Sperm was obtained from 4 stallions which performed 33 to 89 matings ; the best stallion inseminated 184 mares. It was considered that with appropriate dilution of semen and organisation it should be possible to increase the number of mares inseminated with sperm from a single stallion to 300-400. The dose, irrespective of dilution, varied from 15-20 ml. for young mares to 20-25 ml. (and up to 50 ml.) for old or suckling mares, or those with poor quality of vaginal and uterine mucus, or uterine atony. Deep insemination was practised. Semen was diluted with Milovanov's glucose-sulphate diluent (but without peptone), usually 1 : 1 to 1 : 3, but even 1 : 4 and 1 : 5 gave entirely satisfactory results and were recommended for good quality sperm. The average number of inseminations per mare was 3·3 and the average rate of conception 84%, which was lower than for natural mating (94%), but was explained by the fact that many of the mares artificially inseminated were sexually abnormal. The mares were inseminated once a day.

Životkov and Zdanovič (1938) reported data from an artificial insemination station organised to serve 4 studs within a radius of 3 to 5 km. from the station, with a total of 740 mares. Of 5 stallions 2 were used for natural mating, the sperm of 2 others was collected through urethral fistulas and that of the last by the vaginal method. Sperm collected through the fistula survived longer than that collected by the vaginal method, even though the latter was taken from a more fertile stallion. 79 mares were mated normally and 346 artificially inseminated ; the foaling percentage was 87 and 86 respectively. The mean number of inseminations per mare was 3·2. Mares were inseminated in the morning and again after 48 hours (more rarely 24 hours), The usual dose was 15-20 ml. (range 12-60 ml.). It was considered that young and non-suckling mares should be given doses of 15-20 ml., those older and suckling 20-25 ml., and mares with uterine atony or other genital weaknesses 25-30 ml. ; 20-25 ml. of thinner sperm was considered better than 10 ml. of denser sperm. A firm catheter made of plastic substance, which it is stated enabled insemination to be made into the uterine horn, was preferred to the soft rubber one.

Time and Number of Inseminations

The physiological processes in the reproductive cycle of the mare need not be discussed at any length, but some reference must be made to recent work on the time and number of inseminations required, problems which are of greater importance in the mare than in other animals.

Theoretically the best time to inseminate would be a few hours before ovulation, which Lambert and McKenzie (1940) state usually occurs 20 to 40 hours before the end of heat. Day (1940*a*) observed that ovulation occurred in the majority of mares from the last day of oestrus to 2 days before the end of oestrus, but occasionally up to 5 days before the end of oestrus to one day after. A recent study on oestrus, ovulation and related phenomena in the mare has been made by Andrews and McKenzie (1941).

The psychological and physiological signs of heat do not always coincide. Životkov (1933-34) states that as a rule the outer and inner signs of heat correspond but there are about 10-20% exceptions. In false or incomplete heat only the outer signs are found and the cervix is closed. The reverse condition is present in silent heat which occurs in young and shy animals. Some mares exhibit all the signs of heat but do not conceive owing to lack of uterine tone. Patrušev (1935) found that testing mares with a teaser stallion did not always detect mares with latent heat. Kedrov (1938) found, among 734 mares, that heat behaviour at ovulation was either absent or slight in 19%. On the other hand, in some mares heat behaviour was exhibited when there was no follicular maturation, and such mares might remain barren for years, because if tested with the teaser they were always mated at the wrong time. Kedrov states that only 25% to 30% of the mares brought into an artificial insemination station were ready for insemination.

In the opinion of Životkov (1933-34) every mare that shows signs of heat should be examined internally to determine the size, shape, consistence, and degree of opening of the cervix and type of mucus; the last is the most important inner sign of oestrus. Patrušev (1935) considers that the best results are obtained by a clinical examination of the vagina, using a speculum—hyperaemia of the lining membranes, mucous secretion, and the condition of the cervix being regarded as reliable signs of heat. Beljaev (1938) states that the degree of success in insemination depends not so much on the extent of opening of the cervix as on the state of the vaginal walls and mucus.

Kedrov (1938) considers rectal examination the only reliable method of diagnosing oestrus; using rectal examination of the ovaries in conjunction with insemination of 270 mares, he found that the average number of inseminations per conception was not more than 3, as compared with 5-6 when the teaser was used. Životkov, Krakov, and Antonenko (1939) tested mares daily and those found to be on heat were examined rectally to determine the state of the ovaries. Lambert and McKenzie (1940) recommend breeding when there is a large, slightly relaxed ovarian follicle 2-5 cm. in diameter.

Hammond (1938) found that matings on the 2nd day before the end of oestrus gave the best results; the fertility was fairly high as early as the 6th day prior to ovulation, but by the 9th day all matings were sterile. Day (1942) showed that with insemination of approximately 2×10^9 sperm pregnancy resulted from insemination up to 6 days prior to ovulation, but not when made earlier or on the day after ovulation. Salzman (1939) considered that in some instances fertilisation took place when artificial insemination was carried out 12-14 hours after ovulation. Incidentally, imminent ovulation was predicted in only 32% of cases. Životkov (1940) investigated this problem; if mating and insemination of mares during or just after ovulation were effective, this would eliminate the need for repeated insemination and result in a considerable saving of matings and sperm. Two years' analysis showed that the best results, 86-88% fertilisation in over 700 mares, were obtained with insemination prior to ovulation. Insemination during ovulation resulted in a drop of some 12% and 2-10 hours after ovulation in a drop of over 50%.

Neumann and Salzman (1937) decided that, if trained personnel is available, the time of ovulation should be determined by rectal examination and mating or insemination should be timed to fall 10-20 hours before ovulation. In farms where rectal examination is impossible, mares that have foaled should be served on the 2nd day, those that have not foaled on the 3rd day, of heat. Service should be repeated every 24-48 hours. The total number of services during one heat period should not exceed 4-5 and in studs 3-4.

Martynov (1938), on the basis of 2 seasons' experience at 2 studs, decided that the optimum interval between services was 36 hours, starting from the morning of the 2nd day of heat, varying from 24 hours for mares with short periods to 48 hours for mares with longer ones. In general not more than 4 services will be required. Lambert and McKenzie (1940) recommended, if feasible, breeding once daily after the first day of heat in light mares, in draft mares after the 2nd day; if bred only once, on the 3rd day; when a mare is on heat 3 days after breeding, breed a second time.

Artificial Insemination of Other Animals

Pig.—Rodin and Lipatov (1935) reported the results of experimental work carried out since 1931. Best results were obtained with a dilution of $\times 4$ (average fertilisation 70%). The dose of the diluted semen, which ranged from 100 to 150 ml., should vary with the size of the sow, larger animals getting a larger dose. Haring (1937) observed that ovulation begins about 24 hours after the onset of heat and is completed 36 hours, or at the most 48 hours, after the onset of heat. He concluded that the most favourable time for mating is the 2nd day of heat. Rodin and Lipatov obtained a much higher percentage of fertilisation in sows inseminated on the 2nd day. Lambert and McKenzie recommended service late on the 1st day or preferably on the 2nd day.

Dog.—Successful results have been obtained by Alifanov (1933-34) and Hermansson (1935). Freiberg (1935) collected sperm by masturbation, and used the 2nd fraction, 2-4 ml., for insemination. This portion may be divided into 8-10 doses ; the optimum dilution is 1 : 8.

The female to be inseminated must be in the proper stage of heat, preferably, state Lambert and McKenzie, the 11th to the 13th day after beginning to breed.

Fox.—Artificial insemination may be of use for extending the services of valuable male foxes. However, comparatively little work has been done on the technique, and there is still much to be learnt about the oestrous cycle in the vixen. According to Starkov (1933-34) insemination should be done about 6 days after the beginning of heat.

Rabbit.—Artificial insemination has been used to some extent in practical rabbit husbandry (Padučeva and Maksimov, 1934).

Ovulation occurs about 8 to 10 hours after coitus and maximum fertility is obtained from matings 5 to 10 hours before ovulation (Hammond, 1934). Insemination should therefore be made 2 to 5 hours after mating with a vasectomised buck (Lambert and McKenzie), or with an aproned normal buck.

Poultry.—Artificial insemination has not been much used in birds, but, according to Lambert and McKenzie (1940), recent techniques have been developed which make it useful and practical in certain phases of poultry husbandry. It allows a greater use of progeny-tested males; is a valuable means of bringing about fertility of hens kept in batteries ; and is useful in making interspecific crosses or crosses between breeds that differ greatly in size. Black and Scorgie (1942) state that this method might well be utilised by pedigree breeders to obviate the necessity of keeping their birds in small fixed breeding pens which are expensive to erect and difficult to maintain owing to the risk of the ground becoming fowl sick. The breeding hens could thus be kept on free range and artificially inseminated as they are released from the trap nests. This method might also be of use under wartime conditions, they state, to make good any shortage of high-class cockerels that is likely to arise.

Burrows and Quinn (1938) consider that insemination once weekly with 0·1 ml. of good quality semen should result in 80% to 90% fertility. It may be necessary, if the fertility with this procedure is low, to use more semen or to inseminate more frequently, or both. The best fertility is obtained with fresh semen. Jeffrey (1941) states that weekly insemination of 0·1 ml. gave an average fertility of 82%. Bonnier and Trulsson (1939) found that 30% semen gave better results than, and 10% semen as good results as, undiluted semen.

Black and Scorgie (1942) used, at varying intervals, 0·05 ml. semen diluted to 0·1 ml. with normal saline or Ringer solution, in the domestic fowl. There was a tendency for the birds receiving 2 or more inseminations in one week to produce the greater number of fertile eggs.

According to Burrows and Marsden (1938), if turkey hens are properly inseminated with 0·5 ml. good viable semen, twice, at an interval of 3 to 4 days, and if this process is repeated every 3 weeks, from 80% to 90% fertility should be obtained, although the degree of fertility will vary with different hens.

PART III

CHAPTER 9. THE TECHNIQUE OF ARTIFICIAL INSEMINATION

The general procedure consists of (1) the collection of semen from the male, (2) the evaluation of the semen, and (3) injection of the semen into the female (Walton, 1933, 1942 ; Milovanov, 1934). Several methods have been used for collecting semen, but many of them are unsatisfactory and will not therefore be discussed. Collection of semen by the artificial vagina is the common and most satisfactory method for the bull, ram, stallion, and boar. Semen can also be collected from the bull by massage of the accessory genital organs, and from the ram by artificial stimulation.

The artificial vagina consists of an outer cylinder of metal or heavy rubber hose with an inner rubber sleeve which is folded over both ends of the outer cylinder. Water at the proper temperature is introduced into the space between the inner rubber sleeve and the outer cylinder. One end of the artificial vagina is left open and to the other is attached a test tube or bottle for collecting the semen. The inside of the artificial vagina is lubricated. Collection of semen is accomplished by directing the penis into the artificial vagina when the male mounts the female. Ejaculation usually occurs promptly and the semen runs down into the collecting vessel. The advantages of the artificial vagina are : (1) most males work well and readily with it and will continue to do so over a period of years, (2) the ejaculate is collected almost wholly and in a clean and uncontaminated form, and (3) a high quality type of ejaculate is obtained. Other methods of collection will be described under the different species.

The practical application of artificial insemination in animals presents no great difficulties once the procedure is mastered. Emphasis must, however, be laid on the necessity for paying full attention to details. Ideally, the procedure should be carried out, or at least supervised, by specially trained veterinarians or biologists, but it is quite possible to train laymen to use the method successfully. The handling of semen and its evaluation must be done with the greatest care if the best results are to be obtained. Suitable precautions must be observed to prevent the spread of disease and to avoid injury to the male or female. Other important points include the management of males, the frequency of their use and the time relationship of insemination to oestrus and ovulation.

General

The collection of semen and its use for artificial insemination involves certain procedures common to different species. These procedures are sufficiently well known to require only brief mention here.

It is a great advantage if the same paddock or yard is used each time for collection of semen, so that the male becomes accustomed to the surroundings and anticipates service when led to the yard. The yard should be sufficiently large to allow ample room for turning. The floor should be level and non-slippery. Owing to the danger of slipping, smooth concrete floors, especially when wet, are not suitable.

If possible a separate building should be kept solely for artificial insemination work. No disinfectants or strong smelling chemicals should be allowed in this building, as they have a harmful effect on spermatozoa.

Preparation and Cleaning of Apparatus

When new and after use the artificial vagina should be washed thoroughly with hot water to remove French chalk or grease. A little washing soda may be used for this purpose, but the greatest care must be taken to ensure that it is removed completely. The artificial vagina is thoroughly rinsed with several changes of clean, preferably distilled water, allowed to drain, the inner lining swabbed with 65% alcohol and allowed to dry in a dust-free place. The alcohol may be omitted when it is difficult to obtain. When the artificial vagina is used with normal, genitally healthy males it is sufficient if the vagina is thoroughly dry and clean before use. If the artificial vagina is required for immediate use after cleaning, the inner lining may be dried with a clean gauze swab.

All glassware should be clean and dry before use. It is sufficient to wash in hot water, rinse thoroughly in clean water and dry on clean blotting paper in a dust-free place. A hot air oven, if available, is useful. Syringes and nozzles are washed out with cold water, and rinsed with alcohol, followed by clean water. Before use they may be rinsed out with diluting fluid, but it is sufficient if they are perfectly dry.

Before use the whole inner surface of the rubber sleeve of the vagina is smeared with medicinal liquid paraffin, using a clean glass rod to one end of which is fastened a small piece of surgical gauze.

White vaseline may be used for this purpose, in which case it is applied after the apparatus has been filled with warm water. Lambert and McKenzie recommend a good lubricant for general use in artificial insemination. It is made by mixing thoroughly 6 gm. of powdered gum tragacanth with 10 ml. of glycerin ; add to this mixture, stirring continuously, 100 ml. water ; keep in refrigerator to prevent formation of moulds. Castor oil, ground-nut oil, and sim-sim oil have been found perfectly satisfactory by the present author.

Handling and Examination of Semen

As a sudden fall in temperature has a harmful effect on spermatozoa, it is most important to ensure that semen is not suddenly exposed to cold conditions by collecting or placing it in cold vessels, by adding cold diluting fluid, or by sudden reduction of temperature during storage. Suitable methods for the gradual reduction of temperature are referred to under Cattle (p. 126) and Sheep (p. 129). The methods for the detailed examination of semen are given earlier (p. 94).

Selection of Females for Insemination

A thorough knowledge of the female reproductive cycle is essential for the success of artificial insemination. It is most important that females be inseminated at the proper stage of the cycle, that is shortly before ovulation. The time of ovulation is related to time of heat and in general occurs about the end of heat. In practice it is usually impossible to determine exactly the time of onset of heat so one or more inseminations are usually made according to the length of heat. This is particularly important in the mare, which may have very lengthy heat periods (*cf.* p. 117). The best periods for insemination are given under the different species (pp. 125, 129, 131, 132).

Capsules for Gelatinised Sperm

Semen is usually introduced into the female with a syringe, but the Russians have lately used another method, whereby the semen is solidified with gelatin diluents. The solidified semen is inserted in gelatin capsules or in some form of tubing. The effectiveness of capsules is diminished by the use of the vaginal method of insemination, since this involves a great wastage of sperm. Milovanov (1938) therefore used hard cyclindrical gelatin capsules (capacity without lid, 0·7 to 1·0 ml.) for introduction into the cervical canal of the cow.

The capsules were prepared as follows. They were first dried in a drying chamber at 90° to 100° C. for 2 to 8 minutes on a sheet of clean paper, and then immersed in liquid paraffin (m.p. 50° C.) standing in the same drying chamber. The capsules were submerged to the bottom of the vessel with dry forceps, to expel air. The vessel with the paraffin and capsules was taken out of the oven and left at room temperature to decrease to 57° to 68° C. Then the capsules were rapidly taken out of the vessel with the forceps, the paraffin poured out of them, and laid on a sheet of clean paper. During this process, the forceps must not touch their inner walls. After 4 to 5 minutes the lids were placed on the capsules. The lids were not dried, nor covered with paraffin, so as to ensure the swift exit of the sperm from the capsule into the cervix. The capsules were then left in a stand until they were filled with sperm. Capsules thus treated had a capacity of 0·7 to 0·9 ml. The gelatinised sperm was poured into the capsules, the latter covered with the lids and left in a vertical position until the gelatin set.

The time required for the untreated lids to dissolve through moisture in the cervix was 1 to 2 minutes. After 6 to 8 minutes the paraffined part of the capsule also began to dissolve, and after 20 to 30 minutes only dissolving pieces remained. The substance of the capsule, as it dissolved, exercised no harmful influence on the sperm.

Capsules used for transport and injection (vaginal) of gelatinised sperm are made of paraffined paper, reed, or glass (Milovanov, 1938). The dimensions of capsules for gelatinised sperm are :—

	Length.	External Diameter.	Internal Diameter.	Capacity.
	mm.	mm.	mm.	ml.
Cattle . . .	300	10-12	6-7	4-6
Sheep . . .	150	6-8	4-6	1·5-2·5
Horse . . .	450	20-22	15-18	60-80
Pig	500	25-27	20-22	100-150

Glass capsules are made from tubes of the appropriate diameter, cut into the required lengths ; the ends are smoothed in a flame. A paraffined cotton wool plug is used to ensure complete hermetic closure of one end of the tube ; this must be easy to withdraw before insemination. The capsules are filled from a graduated pipette of appropriate capacity.

The technique of insemination depends on whether the capsules are made of gelatin or of other substances (Milovanov, 1938). If they are of glass, reed or paper, the packing is removed and the cotton wool plug withdrawn. The capsule is introduced into the vagina directing it obliquely upwards. The part of the capsule projecting to the outside should not be more than 1 to 1·5 cm. The sperm is now pushed out into the vagina with a wood or glass rod (diameter 3-4 mm. for sheep ; 4-5 mm. for cattle), after which the capsule is removed.

The gelatin capsules should be unpacked just before insemination and introduced into the cervix lid foremost by a capsule gun or by hand. If the cervix contracts and expels the capsule, it should be held in position for 1 to 2 minutes.

Cattle

Apparatus

Artificial Vagina.—The artificial vagina used in Kenya is Walton's model, which has proved very satisfactory. The one disadvantage is the cost of replacing the rubber sleeve.

The Russian model of artificial vagina is used successfully by other workers ; the rubber sleeve for this model is about one-third the price of that in Walton's model. Recently spare rubber sleeves for Walton's model have been difficult to obtain so the following description of a simple type of arti-

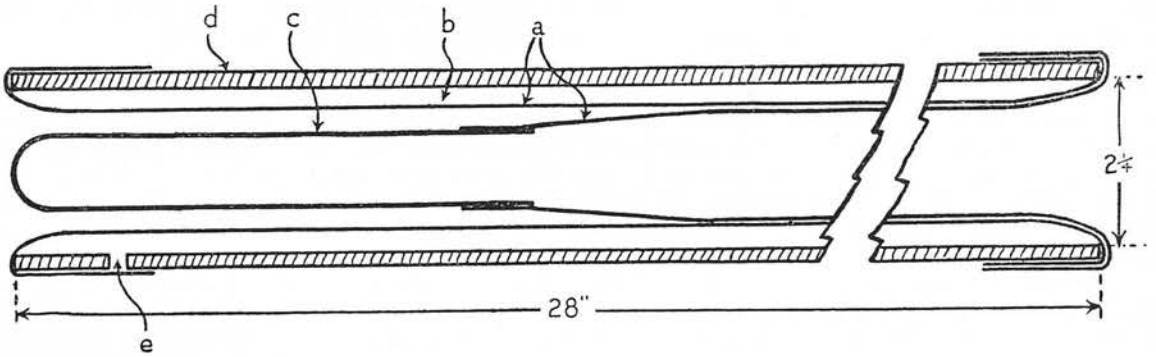


Fig. 6.—Diagram of new-type artificial vagina. (*a*) Rubber liners made from flat rubber band tubing ; (*b*) warm water ; (*c*) test tube ; (*d*) outer tubing made from auto radiator hose ; (*e*) hole for introduction of water. (*From Salisbury and Willett*, 1940)

ficial vagina used by Perry and Bartlett (1939) in New Jersey is given. It consists of a rubber cylinder, 16·5 inches long and 2·7 inches in diameter, fitted with an inner tube, the ends of which are turned back over the ends of the cylinder and held in position with heavy rubber bands or string. Near one end of the cylinder is a hole to admit warm water. This hole is covered by a fold of the end of the rubber tubing or by a screw nut. The large end of a funnel-shaped piece of rubber tube is slipped over one end of the rubber cylinder, and a test tube wrapped in a piece of cloth, to prevent breakage at the time of collection, is fitted into the other end. A very similar type, apparently a modification of a Swedish model, is described by Lambert and McKenzie (1940) ; it consists of a stiff rubber casing 16 by 2¾ inches, fitted with a brass valve, and a thin rubber tube 20 inches long by 3 inches flat diameter which is secured to the outer casing by heavy rubber bands. A thin tapering rubber tube 10 inches long is used to connect the artificial vagina to a glass collecting tube.

Yet another type is described by Salisbury and Willett (1940) which, while somewhat more bulky than the conventional type, is especially useful when semen is being collected in cold weather, for it maintains the proper temperature longer and prevents chilling of the spermatozoa, and thus loss of motility. This type of artificial vagina consists of a water jacket long enough to extend over the test tube, which is held at the temperature of the water. It is made from a 28-inch length of auto radiator hose, 2¼ inches in diameter, and two pieces of 3-inch flat rubber band tubing 0·031 inch gauge (Fig. 6). The water jacket is made as in the conventional artificial vagina. In addition, however, a second inner lining is used which is tapered at one end by cutting out a V-shaped piece

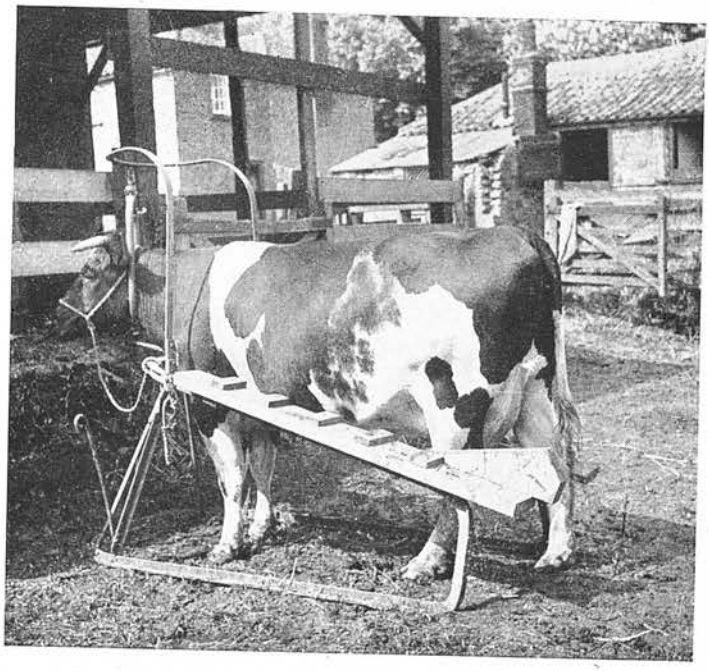
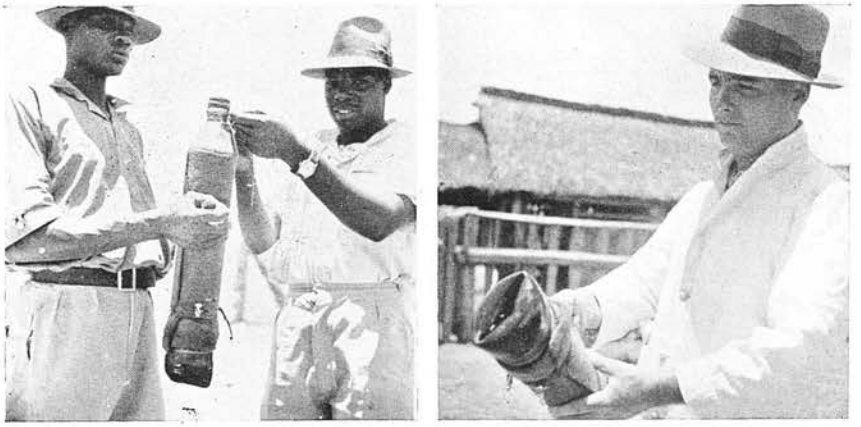


FIG. 7.—Service crate. (Dr. Walton writes that this design has now been improved; the latest model is made by Messrs King, Hertford Works, Hitchin, Herts.)
(*From Edwards and Walton*, 1938)



FIGS. 8 AND 9.—Preparation of artificial vagina (cattle).

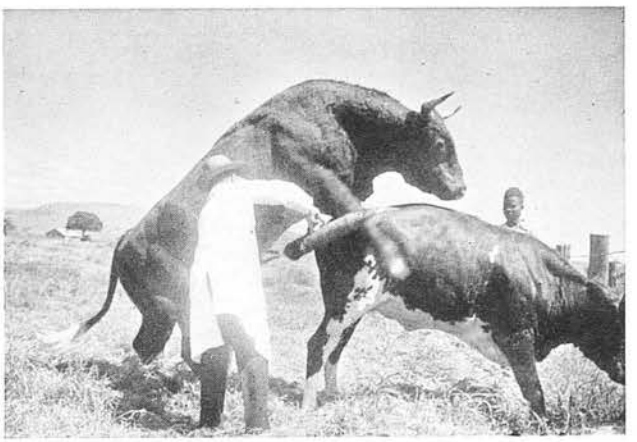


FIG. 10.—Collection of semen (bull).

FIG. 11.—Insemination (cow).

FIG. 12.—Collection of semen (ram).

(*All from Anderson*, 1938*a*)

and cementing the cut edges together with rubber cement, leaving an opening at the end small enough to hold the open end of the test tube. The large end of the inner sleeve is doubled back over the end of the water jacket. Additional advantages are as follows. (1) Occasionally some bulls refuse the conventional type of artificial vagina when thin rubber sleeves are used, apparently due to lack of suitable pressure and cushioning around the opening. This difficulty is obviated with this new artificial vagina, for the two layers of rubber tubing serve as an effective cushion. (2) Because of this cushion effect and the large water capacity, little notice need be paid to the water pressure.

Speculum.—A small size of speculum is required for heifers and small breeds. A two-blade speculum is best for this purpose, but for larger animals a three-blade one is best (Polansky's 14½-inch blade). A speculum of this type is comparatively expensive and it is possible to use simpler methods :—

A metal tube 1½ to 2 inches in diameter and about 12 to 14 inches long, or a strong glass tube of similar size can be used. Another suitable type is described by Barron (1941). A speculum can be dispensed with by using the hand to guide the nozzle into the cervix (see under " Insemination ").

Syringe.—The common type is a glass syringe of 2 ml. or 10 ml. capacity, to which is attached a vulcanite nozzle 18 inches long. The glass tip of this syringe is very vulnerable and the joint between the syringe and nozzle can be strengthened with sealing wax. Another method to ensure rigidity of the syringe and nozzle is to attach a celluloid knitting needle to them by rubber bands (Lambert and McKenzie, 1940).

A glass capillary tube, 16 inches long with an internal diameter of 1-2 mm. and an outside diameter of 6 mm. can be used instead of the vulcanite nozzle. The ends should be well rounded. It is connected to the glass syringe by a short piece of rubber tubing.

Extreme economy of semen is not usually important unless in cattle breeding associations using artificial insemination. The wastage of semen which occurs in the nozzle may be reduced by using liquid paraffin in the syringe as mentioned under " Dilution of Semen " (p. 124), or by using a special syringe with the barrel at the front end of the inseminator. This type of syringe is better than any other, for it economises the semen, is less liable to breakage, and is easily cleaned.

Control of Bull and Cow

For collection of semen on farms a cow on heat will usually be available—preferably an older cow which has been handled a lot and stands quietly. The cow is tied to a post with a halter or with a rope round the horns. The ground should be level and not slippery. When testing the fertility of bulls by semen examination and when artificial insemination is used in cattle breeding associations a cow on heat may not be available. A cow not on heat can, however, be used successfully provided she is tied or held to prevent her moving about. For the regular collection of semen a cow can very soon be trained to stand for the bull. A very suitable service crate for holding the non-oestrous cow has been described by Edwards and Walton (1938) (*see* Fig. 7). A dummy cow can also be used for regular collection. Bulls should be led up to the cow either on a halter or on a pole or rope attached to the nose ring.

Preparation of Apparatus

The preliminary steps in the preparation and care of the apparatus have already been described. After lubricating the inner surface of the rubber sleeve of the vagina, water heated to 45° C. (113° F.) is filled into the cup end of the vagina. The free end of the rubber lining is then folded back and tied in place with string (*see* Figs. 8 and 9).

The important point is to have the artificial vagina at the proper temperature at the time of collection. The temperature at which water is filled into the vagina depends partly on the type of vagina used, external conditions, and the time that elapses between the preparation of the apparatus and the actual collection of semen. With the Russian model of artificial vagina, Walton recommends filling in water at a temperature of about 55° C. The artificial vagina may be filled, holding it horizontal, by means of the tap.

The small glass bottle, into which about 2 ml. of liquid paraffin is placed, is then inserted into the small end of the artificial vagina and held in place by the metal cup, which is screwed on. The pressure is best adjusted when filling in the warm water and can be altered either by filling in more water, blowing air into or by running out water through the tap.

A suitable standard pressure can be obtained in the Russian model by holding the artificial vagina vertically with the mouth downwards ; on opening the stopper any surplus water will run out. The stopper is then wiped with a clean cloth and any drops of water removed from the artificial vagina.

The temperature of the artificial vagina must always be taken immediately before collection of semen by inserting a clean dry thermometer into the end farthest away from the cup. At the time of collection the temperature should be between approximately 39° C. and 43° C. (102° F. and 111° F.); it should not be above 44° C. as higher temperatures are harmful to spermatozoa. It may be as low as 36° C. (97° F.) provided this temperature does not prevent ejaculation.

Collection of Semen

Artificial Vagina.—The collector stands on the off-side of the cow, holding the artificial vagina by the handle in the right hand. When the bull mounts, the artificial vagina is raised close to the flank of the cow, behind the bull's foreleg, with the opening directed towards the penis, at an angle corresponding to the probable line of erection and thrust of the penis, *i.e.* at about 45°. The penis is directed into the artificial vagina with the left hand which grasps the sheath (*see* Fig. 10). The penis should not be touched by the hand, as this may cause retraction. When the penis enters the artificial vagina the bull thrusts vigorously forward and upward and ejaculates. Sometimes this may not happen immediately the penis enters the artificial vagina and occasionally a bull will mount the cow two or three times before ejaculation occurs. A bull does not ejaculate properly unless he has this final upward thrust. When the bull has ejaculated he dismounts from the cow. The artificial vagina should be tilted with the bottle end lower than the open end so that the semen runs into the bottle. It should be held in position at the end of the sheath for a moment to catch any final drops of semen.

Massage.—Semen may be obtained by massaging the accessory genital organs of the bull (Miller and Evans, 1934) (*see* Fig. 13). The hand is inserted from 7 to 10 inches into the rectum and the seminal vesicles massaged with backward strokes. A turbid fluid flows from the prepuce, containing only epithelial cells in the majority of cases. The ampullae of the vasa deferentia are then massaged in a similar manner. From the ampullae is obtained a turbid fluid rich in spermatozoa. The semen is collected in a wide mouthed bottle or test tube, using a small glass funnel. This method requires considerable experience and not all bulls respond well. The ejaculate may sometimes be retained in the sigmoid flexure of the penis (Lambert and McKenzie, 1940); to avoid this the operator should straighten this flexure with his hand after massaging the ampullae. This method should only be used when a specimen cannot be obtained with the artificial vagina. It is useful with valuable breeding bulls which are unable to serve cows in the normal manner because of injury. On the other hand, as shown elsewhere, semen obtained in this way is of poorer quality than that obtained with the artificial vagina (*see* p. 7).

Handling and Examination of Semen

The metal cup at the end of the artificial vagina is now unscrewed and the bottle containing the semen removed. The handling of the semen and its examination are described more fully earlier (p. 94). The semen should be examined immediately after collection; if examination is delayed, and suitable precautions to avoid a fall in temperature are not taken, the activity of the spermatozoa may be reduced. If the semen is not to be used immediately or within half an hour at the longest, the procedure under storage must be followed. It is, however, highly desirable that the semen after collection should be exposed to known temperatures (see under general procedure and storage, pp. 121, 126) rather than to room temperature which may vary considerably with the time of day and season of the year.

For purposes of artificial insemination the estimation of motility is sufficient. A more complete examination should be made when purchasing new bulls and from time to time afterwards. The writer regards 70% motility as the arbitrary permissible limit for insemination; it is quite possible that semen with a lower motility may prove fertile, but the aim should be to use only the very best specimens of semen, and ejaculates with a motility less than 70% are not advised. It is a comparatively easy matter to distinguish motility higher or lower than 70%.

Dilution of Semen

On most farms there is usually sufficient semen in one ejaculate to inseminate all the cows that come on heat at once, without having to dilute it. But sometimes, because of the large number of cows to be inseminated or the desire to economise semen, it may be necessary to dilute the ejaculate. Various solutions can be used for this purpose but it is recommended that Phillips' (1939) egg-yolk-phosphate medium or the egg-yolk-citrate buffer (Salisbury, Fuller, and Willett, 1941) be used. These mixtures have the added advantage that they are suitable for storage of semen (*see* p. 126).

To dilute the semen, the volume of the ejaculate is measured and the appropriate amount (*i.e.* an equal or two or three volumes) of diluent added drop by drop and mixed gradually with the semen

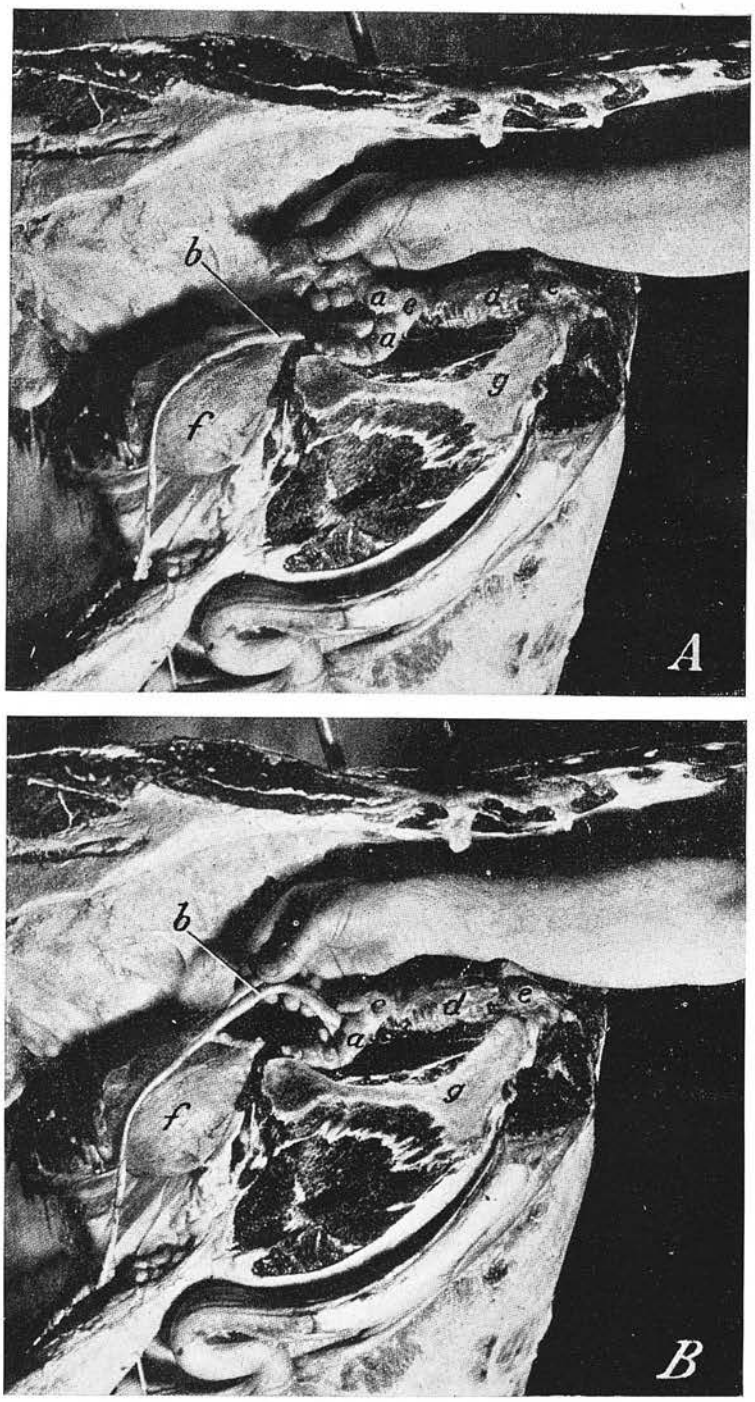


FIG. 13.—Position of the genital organs of the bull and method of manipulating them : *A*, massaging the seminal vesicles ; *B*, massaging the ampullae of the ductus deferens. *a*, seminal vesicles ; *b*, ampullae ; *c*, body of prostate ; *d*, pelvic urethra ; *e*, bulbo-urethral (Cowper's) glands ; *f*, urinary bladder ; *g*, pubis.

(*From Miller and Evans*, 1934)

by gentle inversion of the tube. It is highly important that the diluting fluid should be warmed to about 30° C. (86° F.), or the temperature of the semen, before being added. For use in cattle breeding associations, semen should be diluted according to the concentration of spermatozoa and not just by adding so many volumes of diluting fluid to one volume of semen, in order that the fullest use be made of available semen.

The semen is now taken up in the syringe and air bubbles got rid of. It is sometimes an advantage to draw up 1 ml. of liquid paraffin into the syringe before taking up the semen. This method ensures that all the semen is expelled and the residual fluid in the syringe and nozzle consists of liquid paraffin. Care must be taken, however, to see that semen and not liquid paraffin is injected into the cow.

Selection of Cows for Insemination

Cows to be inseminated must either be on heat at the time of insemination or have gone off heat a few hours previously. The procedure in Kenya is to pick out cows in the morning and evening, but they are inseminated once only per day in the morning. An appreciable number of cows picked out the previous day will hold to insemination the following morning, although they are then no longer on heat.

It is not ordinarily a difficult matter to detect cows on heat in temperate countries, but in Kenya cows have shorter and less intense heat periods (Anderson, 1936), so those on heat may therefore be easily missed. The detection of heat therefore assumes more importance in Kenya than elsewhere and for this reason the methods used are referred to here in some detail; if cows are missed the effect on the birth rate is liable to be just as serious as that of genital disease.

During the day-time it is a simple matter to pick out cows properly but a certain number of them may start their heat periods after dark. Probably most of the cows which start their heat period after midnight will still be on heat at dawn the following morning and will be detected in the usual way. Those starting between nightfall and midnight may, however, be off heat by the morning and will be missed unless special steps are taken to detect them. This has been attempted by observation and by using raddle on the teaser bull as with rams, but so far no satisfactory solution to this problem has been found.

A vasectomised or " teaser " bull is a very reliable method for detecting a cow on heat when the herd is large. A cow coming on heat is easily recognised by the bull following her about and from time to time, particularly as the onset of oestrus approaches, attempting to mount her, but unless she is actually on heat she will not stand for service. Similarly a bull will follow a cow that is going off heat. The end of heat is a gradual transition from receptiveness to one of non-receptiveness. The signs of heat are best displayed when the bull or other cows are present. It is a curious and interesting fact that cows tend to come on heat in groups, an occurrence which facilitates artificial insemination. A cow at the beginning of heat very often becomes restless; she twitches her tail frequently and raises it, together with the tail head, lowering her hips and the small of her back. When on heat she may lick the bull and when separated from other cattle will low.

When heat is short and of slight intensity, the external signs of oestrus are often scarcely recognisable unless the speculum is used. Usually, however, a flow of mucus, at first clear and fairly fluid, comes from the vulva. Later it contains yellowish cheesy lumps and afterwards it becomes thicker and whitish. This flow of mucus is normal and is to be distinguished from abnormal discharges due to infection. In heifers there is very often a flow of bloodstained mucus 2-3 days after the beginning of heat. About 6 days after the beginning of heat the vagina is fairly dry. These changes may be seen in the mucus, although heat may not have been detected because of short heat periods. They suggest that the cow may be on heat again after the normal cyclical interval.

Insemination

The cow is secured in a stall or bail and her legs tied if necessary. It is an advantage to have a fairly narrow stance so that the cow cannot move about too much. The bail should, if possible, be arranged so that the light, at the usual time of insemination, will be directly behind the cow. This light is usually sufficient to illuminate the cervix without the use of an artificial source. If extra light is required a head lamp or a lamp attached to the syringe nozzle may be used. The vulvar region is cleaned with a wet sponge or cloth and then dried. A disinfectant must not be used for this purpose.

The speculum is now inserted into the vagina with the handle held to one side; the speculum is then turned round so that the handle points downwards and the blades opened. The speculum is moved about until the cervix is exposed.

The tip of the syringe nozzle is then introduced about 1½ cm. into the cervix and 0·5 ml. of semen is slowly injected, so that it all remains in the cervix. The syringe is then withdrawn, followed by the speculum. The blades of the speculum must not be closed in the vagina, or else the vagina will be nipped between the blades. During the insertion of the speculum the cow may arch her back and strain. This can usually be stopped by pressing the vertebral column in the middle of the back. (*See* Fig. 11).

If another cow is to be inseminated the nozzle must be wiped with cotton wool soaked in 65% alcohol, and dried with a dry cotton wool swab. The speculum must be washed, dried, swabbed with 65% alcohol and dried again. This procedure is repeated before each cow is inseminated.

Alternatively the cervix may be found by hand either per rectum or vagina. With the former method the cervix is fixed with one hand by way of the rectum and the nozzle is guided with the other through the vagina into the cervix. With the latter method the cervix is grasped with one hand in the vagina and the nozzle directed into the cervix. Great care must be taken to avoid the possibility of carrying infection from one cow to another with this method. The preferred method in artificial breeding associations in America is to use a rubber glove and sleeve and hold the cervix through the intestinal wall so that the inseminating tube can be guided through the cervix. Use of the speculum does not permit the inseminating tube to be inserted through the cervix (Trimberger, 1942). (*See also* p. 141).

Maruškin and Sivokonj (1939) used medicinal capsules, treated with paraffin, containing 150×10^6 sperm. The capsules were kept for 15 minutes at room temperature to ensure solidification of the gelatine. The capsules were introduced into the cervix and held there by hand for 5 minutes to ensure dissolution of the lid (*cf.* pp. 116, 121).

Storage of Semen

Two essential conditions for the successful storage of bull semen are (1) gradual cooling, and (2) the addition of Phillips' (1939) egg-yolk-phosphate medium, or the egg-yolk-citrate buffer (Salisbury, Fuller, and Willett, 1941). The method which has given good results with the author is as follows :—Semen is collected in the usual way with the artificial vagina, the temperature of which should be carefully checked. The semen bottle and all glassware used for handling the semen, as well as the liquid paraffin, should be warmed to a temperature of 30° (86° F.), *e.g.* by placing them in a pan of water at this temperature or in a temperature-controlled oven. After collection, the semen remains in the bottle at this temperature for a few minutes, during the estimation of motility. If the surroundings are cold, it may be worth while, in order to avoid harmful effects of temperature shock, to collect the semen in a shed rather than in the open air.

To one part of semen is added from one to three parts of the egg-yolk-phosphate or citrate medium (*see* p. 71 for formulae), warmed to 30° C. before use. The semen is now placed in Pyrex test tubes which are wrapped in cotton wool. Liquid paraffin is added to the tubes, which are corked and sealed with candle wax. The tubes are placed for 15 minutes in a thermos flask at 25° C. (77° F.), then kept for 15 minutes in a flask at 20° C. (68° F.) and for ½ to 2 hours at 15° C. (59° F.) and finally stored at 10° C. (50° F.). The maintenance of this temperature can best be accomplished by keeping the flask in a refrigerator, but the temperature should be taken twice daily and adjusted to 10° C. when necessary. With this method of storage about three-quarters of the ejaculates retain a high motility till the 72nd hour and a smaller number until the 96th and 120th hour.

Gunsalus, Salisbury, and Willett (1941) found that aseptic methods must be practised to produce a yolk-phosphate without undue contamination. Their method is as follows : fresh eggs are obtained from pullorum-free flocks and immersed in normal NaOH or 70% alcohol to sterilise the shell. The eggs are then broken and the yolk removed, using sterile glassware, and mixed with sterile buffer. Since bacterial growth is greater at 10° C. than at 5° C. the latter temperature is preferred by them for storage.

Sheep

Apparatus

The usual apparatus requires little special mention. Instead of an ebonite nozzle for the syringe, 10-inch glass tubes of ⅛ inch inner diameter, that have been well rounded at one end and slightly tapered and rounded at the other, may be substituted ; these may be attached to the syringe by short pieces of rubber tubing (Lambert and McKenzie, 1940). A suitable speculum may be made from a Pyrex test tube 6 inches long with an inner diameter of ¾ inch, the closed end of which has been cut off (Lambert and McKenzie).

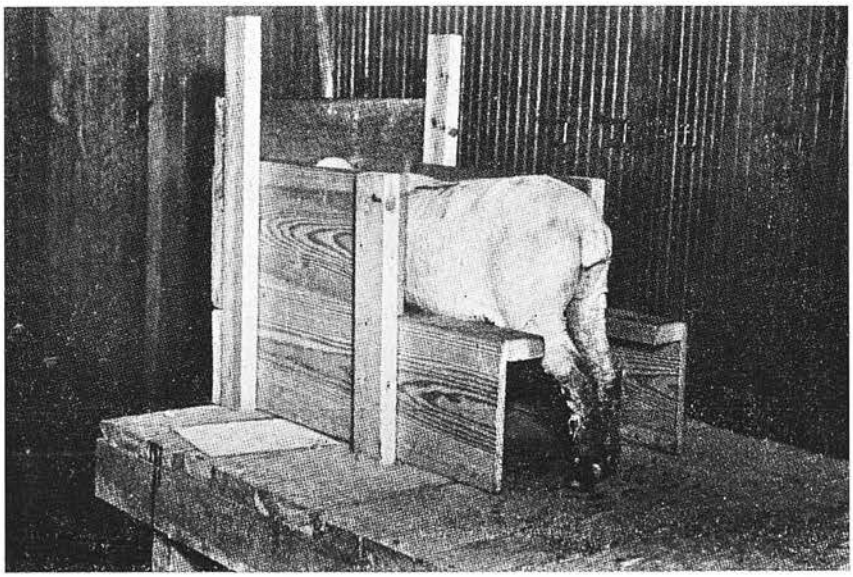


FIG. 14.—A ewe in the service crate.

(*From McKenzie and Berliner*, 1937)

FIG. 15.—Gunn's ram restraint table showing top vertical.

FIG. 16.—The same showing top horizontal.

(*From Gunn, Sanders and Granger*, 1942)

Electrical Apparatus.—A method for the collection of semen by electrical stimulation has been devised (Gunn, 1936 ; Gunn, Sanders, and Granger, 1942). Alternating current, either from a town supply or from batteries, may be used. Alternating current from an electric plant may be rendered suitable by passing it through the primary of a small transformer of about two ampères' capacity and tapped in the secondary at voltages, say, from 5 or 10 volts by fives or tens to 50 volts. The apparatus used by the writer works from a 6 volt battery and is thus portable and independent of an electric plant. When a battery is used the current is passed through a small interrupter and reverser and the resulting intermittent current of reversing polarity is passed through a voltmeter (or definite tappings from the transformer may be used) and a milliammeter, before continuing to the animal. A sliding resistance is used for controlling the milliampères.

The rectal electrode consists of thick copper wire, insulated throughout its length by binding it with adhesive waterproof tape, except at the free end. Another type consists of an ebonite rod with a large brass end length, 10 inches long (Fecundazione Artificiale, 1938). The rectal pole should be quite smooth and have a rounded end. For the other electrode which is inserted into the longissimus dorsi muscle, Gunn uses a stout needle soldered to one of the insulated wire leads. Other types are : a wire flattened at one end so that it has a surface of about $\frac{1}{4}$ inch in diameter (Lambert and McKenzie, 1940) ; or a metal ball covered with bucksin, treated before use with physiological saline ; or a roller formed by two cylinders, covered with buckskin, each being about $1\frac{1}{2}$ inches in diameter and $\frac{3}{4}$ inch wide, with $\frac{7}{8}$ inch space between them (Fecundazione Artificiale, 1938). There is little, if any, difference in the results obtained with the different electrodes, but when frequent collection is to be made by this method it seems preferable to use a lumbar electrode that does not require to pierce the skin.

Control of Ewe and Ram

Ewe.—When training a ram it is advisable to use a ewe on heat, but after he has become accustomed to the procedure one not on heat may be used. A quiet ewe used to being handled should be chosen. A ewe can be trained for this purpose. She should be held by the shoulders and neck to prevent her from moving about. A service crate about 18 inches high may be used for holding the ewe, but is not essential (*see* Fig. 14).

Ram.—The ram should be quiet and easily handled. Young rams are often wild and, though sexually virile, pay no attention to a ewe on heat, particularly in strange surroundings. If such rams are handled frequently and are exercised on a halter they will soon settle down. Young rams that have not served before should be allowed to serve two or three ewes before semen is collected with the artificial vagina. When the ewe and collector are in position, the ram should be led up to the ewe and should mount her almost immediately. A second collection can be made after an interval of 10 to 15 minutes.

For collection by electrical stimulation, the ram is secured on his side on a suitable table or bench. The most favourable position of restraint is in extension, as the general body reaction is towards extension. The forelegs are therefore tied together and held or secured in a forward position and the hind limbs are similarly secured and extended backwards. The head is also fixed in an extended position (*see* Figs. 15, 16, 17).

Preparation of Artificial Vagina

This is done in the same way as for the bull. The correct distension, which is learnt by experience, is obtained by means of the tap on the side of the artificial vagina. An estimation of the correct pressure can be made by inserting the thumb into the lumen ; it should pass in easily but a slight pressure should be felt (Walton). The correct temperature at the time of collection is from 38° C. (100° F.) to 42° C. (108° F.) ; below this ejaculation may be inhibited.

Collection of Semen

Artificial Vagina.—The artificial vagina is held against the flank of the ewe, and when the ram mounts, the penis is directed into it with the left hand applied to the sheath (*see* Fig. 12). The penis must not be touched by the hand. When the penis enters the artificial vagina the ram should thrust upwards, and ejaculate semen into the upper part of the artificial vagina or into the bottle. The artificial vagina is then held vertical, bottle end downwards, to allow the semen to run into the bottle. Some rams are slower than others in ejaculating, and some may mount the ewe two or three times before they have a final thrust and ejaculation.

Electrical Stimulation.—In woolled breeds, the wool should be clipped over the lumbar region

and along the sheath. Before collection the end of the sheath should be wiped with cotton wool. The penis is drawn out of the sheath and held with the urethral process or glans penis within a test tube (when semen is ejaculated in small amounts it tends to dry on the walls of the test tube ; a Cambridge pattern glass cup containing liquid paraffin is rather better than a test tube). In some rams it may be rather difficult to get the penis out, and straightening the sigmoid flexure may help. The penis may be kept extended by wrapping a piece of gauze round the protruding end.

The rectal pole is now inserted about 10 cm. into the rectum. This should be done with great care to avoid injury to the bowel. Perfect contact should be ensured by firm but gentle upward pressure of the uninsulated end of the rectal pole and the upper part of the bowel wall to avoid injury

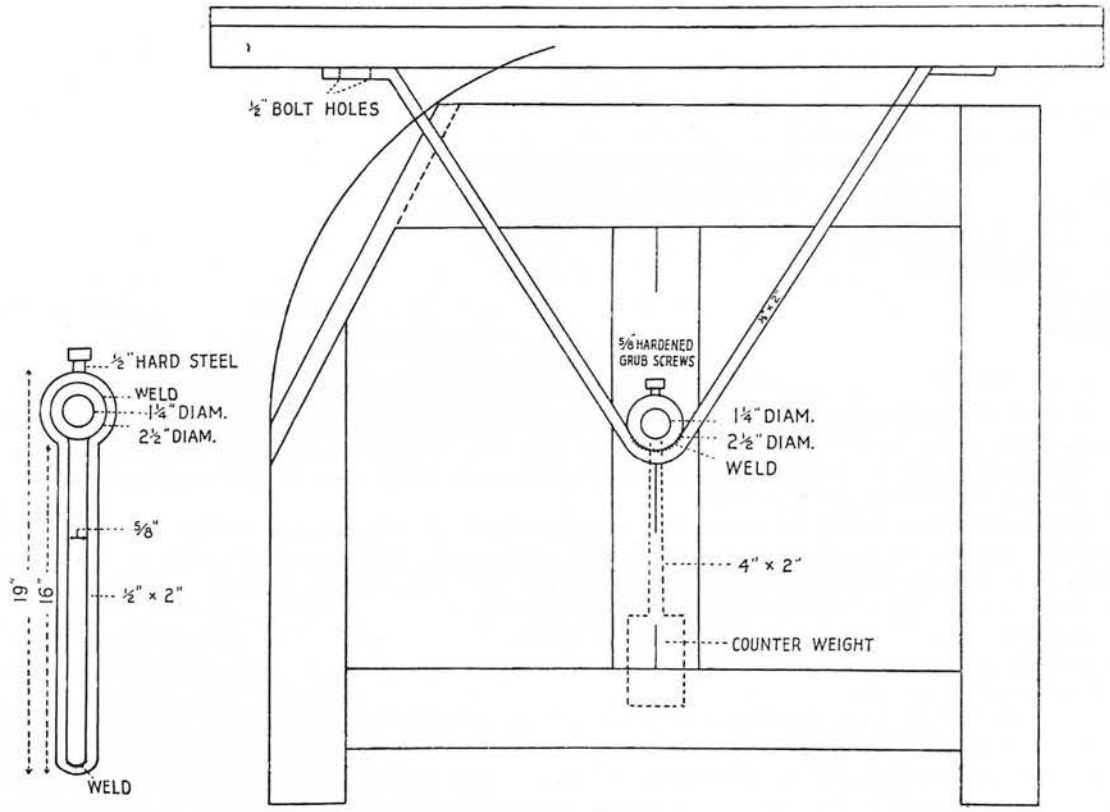


FIG. 17.—Side elevation of Gunn's ram restraint table.
(*From Gunn, Sanders and Granger*, 1942)

to this by sparking as a result of imperfect contact. Before insertion of the rectal pole a small amount of physiological saline solution may be injected into the rectum to ensure good contact.

The current is now switched on and the other electrode is firmly applied to the skin in the region of the 4th lumbar vertebra. The skin should first be moistened in this region. A series of stimuli at 30 volts is now applied for 5 or fewer seconds with corresponding intervals. The stimuli may be applied 10 to 20 times. Usually semen containing the highest number of spermatozoa comes away after the first few stimuli. With further stimuli the semen becomes thin and watery and contains fewer spermatozoa. There is considerable variation between rams, and in the same ram from time to time, in the number of stimuli required to produce a good ejaculate.

The position of the rectal electrode in relation to the lumbar electrode is of considerable importance. During the first few stimuli the rectal pole may be somewhat approximated to the lumbar pole and then returned to its sacral site to ensure complete ejaculation.

Bacteriologically sterile semen can be collected by withdrawing the penis from the sheath, cleansing it with sterile water or physiological saline solution and allowing only the urethral process to enter the test tube. The first spurt of semen should be discarded as this helps to wash out any contamination from the urethra.

This method has no harmful effects on the rams. There may be some motor inability of the hind quarters, varying with the number of stimuli and the current used, but these effects soon pass off. The author has used this method of collection on many occasions with very successful results. Collections may be made from the same ram at daily intervals or every second day over long periods, as Lambert and McKenzie (1940) have stated, without any harmful effects. Collection by this method is very useful when semen cannot be obtained with the artificial vagina. Considerable experience and care is required in its application and it should only be used under expert supervision.

Handling and Examination of Semen

The general procedure is the same as for the bull. It is important to avoid any sudden fall in temperature which may be harmful to the spermatozoa. The method followed by the author when the semen is used within ½ to 1 hour of collection is to keep the semen at 25° C.-30° C. during the examination for motility and then at 20° C. during insemination. If the semen is not to be used within this period it should be treated as for storage.

Undiluted semen, examined microscopically, should show characteristic turbulent wave movements. Only ejaculates which show this very active motility should be used for insemination.

Dilution of Semen

The appropriate amount of the diluting fluid (*e.g.* up to 3 volumes) is added drop by drop and mixed gradually with the semen by gentle inversion of the tube. The diluting fluid should be warmed to about 30° C. (86° F.) before being added to the semen. It is advisable to examine the motility of the semen after addition of the diluent and when insemination is completed.

Selection of Ewes for Insemination

Ewes are picked out by vasectomised rams which are run with the ewes morning and evening, and ewes are inseminated morning and evening as long as they are on heat. This procedure is the one that gives best results with high-grade Merino sheep in Kenya. Care should be taken to ensure that these rams are fully virile during the breeding season ; they may with advantage be changed every 2 to 3 weeks. The use of " raddle " on the rams may help to pick out the ewes. The ewes should be upset as little as possible during the inseminations ; for example, semen should be taken out to the ewes, rather than the ewes brought in any distance for insemination.

Insemination

The ewe is stood on a table or placed over a rail, 3½ to 4 feet high. Alternately, a crate may be used standing 18 to 20 inches high and mounted on an axle, so that the hind end of the ewe can be swung round to the inseminator and tilted so as to lower the head of the ewe.

The speculum and syringe nozzle are wiped with a cotton wool swab soaked in 65% alcohol and then dried with a dry swab. The speculum is inserted into the vagina with the left hand, with the flat side of the blades vertical. It is then turned round so that the handle points downwards, opened and the cervix exposed. The tip of the syringe is inserted about 1 cm. into the cervix and 0·2 ml. of semen injected. The syringe and speculum are then withdrawn and disinfected before inseminating another ewe.

Storage of Semen

The great importance of temperature conditions for the successful storage of ram semen has been established by Chang and Walton (1940). Semen is handled at 25° C.-30° C. and the temperature is then reduced gradually to 20° C. in about 25 minutes. Thereafter it is cooled gradually in stages of 5° C. with an interval of 2 hours between the stages to a temperature of 1° to 3° C. Such slowly cooled semen retained 74% of its original respiratory activity for 6 days. If semen is to be stored for 24 hours, 5° C. is a more favourable temperature than 1° C. Storing the whole quantity of semen in one tube gives better results than storing in small quantities.

HORSE

Apparatus

The Cambridge pattern of artificial vagina, similar in design to the Cambridge bull artificial vagina, is the type usually used (Walton, 1938*a* ; Day, 1940*b*).

Another type is the Missouri-U.S.D.A. model which consists simply of a rubber tube about 18

inches long and 7 inches in flat diameter, with a rubber ring placed in the open end and the other end narrowed down to stretch over a bottle (Lambert and McKenzie, 1940) (Fig. 18). To develop the proper pressure a second rubber tube of the same size is drawn over the first and the two tubes are then vulcanised together at each end. A tyre valve is placed in the outer tube so that air may be pumped between the two tubes. The rubber tubes are enclosed in a leather casing to which a handle is attached. The 3-inch rubber band placed around the inner tube near the open end is an essential feature of this model. This band simulates the sphincter muscles of the mare and helps materially in making collections. Just before use the apparatus is warmed by pouring hot water through it and flushing with either physiological saline solution or a semen dilutor. Flushing with saline is unnecessary if the valve is large enough to pour a quart of hot water through it to warm the apparatus. As much of the flushing agent as possible should be shaken out before attaching the collecting device.

Control of Stallion and Mare

Both the stallion and mare are handled in the usual way for service. The mare should have a groom at her head and service hobbles on, and a twitch is necessary. The mare should be on heat and should be tried over the bar or gate, as for ordinary service, just before collection.

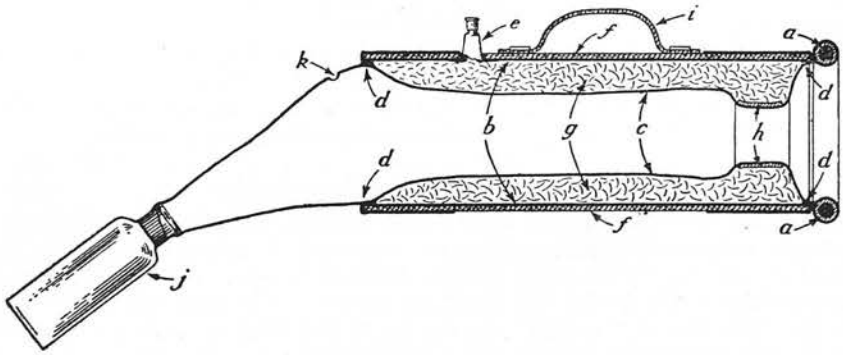


FIG. 18.—Longitudinal section of the Missouri-U.S.D.A. model artificial vagina for the horse. Length 18 in., 7-in. flat diameter rubber tubing; *a*, entrance ring made by enclosing section of ½-in. garden hose; *b*, outer tube; *c*, inner tube; *d*, points at which the outer tube is vulcanized to the inner tube; *e*, air valve; *f*, leather casing to give support and rigidity; *g*, air space between inner and outer tube which allows for the adjustment of pressure; *h*, the " sphincter " rubber band, made of 3-in. flat-diameter tubing 1½ to 2 in. broad; *i*, handle grip attached to leather casing; *j*, 8-oz. collecting bottle; *k*, air vent to prevent ballooning.
(*From Lambert and McKenzie*, 1940)

Preparation of Apparatus

In the Cambridge pattern artificial vagina the rubber sleeve is turned inside out and smeared with vaseline and then turned right side out and worked between the hands to smear the vaseline evenly. The sleeve is then placed in the metal cylinder, the narrow end of the lining is turned back over the casing and bound in position with string. The bottle is placed in position and the cap screwed on.

The cap is placed on the ground with the artificial vagina held mouth upwards. Holding back the lining, the space between the lining and the metal cylinder is filled to the brim with water at about 50° C. which warms the apparatus in about half a minute. About half of the water is now poured away, the lining is turned back over the mouth of the metal cylinder and fixed firmly with the metal band or string. The apparatus should now be water-tight and the internal temperature about 45° C.

The pressure required depends on the size of the stallion and extent of dilatation of the penis. The pressure is adjusted by blowing air through the tap or by letting out water. To test the pressure, place the forearm with clenched fist in it. For the Thoroughbred stallion a gentle pressure should be felt. It is better to have the pressure rather too great than too slack as stallions appear to be more vigorous with greater pressure (Day, 1940*b*). For heavy draught horses little pressure should be felt when the closed fist is inserted.

Collection of Semen

Two assistants are required to hold the artificial vagina. Both stand on the near side. The first assistant holds the artificial vagina with his left hand and guides the penis in with his right hand.

The second assistant holds the upper end of the artificial vagina high up on the flank of the mare at an angle of 45°.

The stallion is led up to the mare and allowed to mount. The groom in charge of the stallion should stand well forward when the stallion mounts. Usually the stallion makes vigorous thrusts of the penis until it is inserted well into the artificial vagina ; if he tends to withdraw or the thrusts become less vigorous, the artificial vagina should be pushed down against the penis. If this does not restore activity to the stallion, the pressure should be increased before a second trial. The pressure may, however, become too great at the moment of ejaculation when the penis becomes distended. This can be noted by the first assistant who can feel the distension of the rubber lining round the mouth of the artificial vagina with his right hand. He should instruct the second assistant to open the tap a little and relieve the pressure. When the stallion ejaculates, the first assistant can feel with his right hand the peristaltic waves of the urethra passing up the base of the penis. When these waves have passed, the penis relaxes and the stallion prepares to dismount. The second assistant now lowers his end of the apparatus and both assistants retire together leaving the stallion to back off the mare.

Handling and Examination of Semen

The ejaculate of the stallion varies in volume from 50 to 200 ml. and consists of two portions : (1) the sperm-serum which is ejaculated first, a watery somewhat opaque fluid containing in the normal fertile animal a large number of active spermatozoa, followed by (2) a viscous mucous secretion from the seminal vesicles, containing no spermatozoa.

The second portion is best removed as soon as possible, for it tends to block the syringe and soon becomes penetrated by spermatozoa. The semen is placed in a small glass bowl containing sufficient liquid paraffin to cover it and protect it from exposure to the air. The bowl is then tilted until the semen almost runs over the edge, and the thick vesicular secretion is picked out with a thermometer or glass rod and allowed to run over the edge into a separate bowl.

Selection of Mares

The mare should be tried with the stallion to be sure that she is on heat. Insemination should be performed towards the end of heat as far as possible. Since mares may stay on heat a considerable time they should be re-inseminated at 3-day intervals until the end of heat.

Insemination

Service hobbles are placed on the mare and if necessary a foreleg held up or a twitch applied. The mare is stood facing a dark or shaded wall with her hindquarters to the light. The vulva should be wiped clean and dried. The speculum is inserted and the cervix exposed. The syringe is filled with sperm-serum and a minimum of 10 ml. is injected ; if sufficient semen is available double this quantity can be given. Day has obtained good results with 30 ml. The nozzle of the syringe is inserted through the cervix, gently rotating it from side to side if difficulty is experienced, and the semen is slowly injected into the uterus.

A speculum can be dispensed with by inserting the hand into the vagina and guiding the nozzle into the cervix ; difficulty in working the nozzle through the cervix can usually be overcome by massaging the cervix for a few minutes (Lambert and McKenzie).

A simple and convenient method of insemination, using either a $\frac{1}{2}$-ounce or 1-ounce gelatin capsule, is mentioned by Lambert and McKenzie (1940) ; 10 to 30 ml. semen is poured into the capsule, the capsule closed, and then carried into the vagina without delay by the hand and placed well forward in the cervix. The hand and arm of the operator should be clean and well lubricated. Infection can be kept to a minimum if a rubber glove and obstetrical sleeve are used.

Storage of Semen

Storage is not yet practicable for periods longer than 4 to 6 hours. If the semen is not used immediately it should be covered with liquid paraffin and slowly cooled to 10° C.

Pig

Apparatus

Semen may be collected by a very simple type of artificial vagina in which no special provision is needed to keep the apparatus warm during copulation. One simple type described by McKenzie (1931) consists of a soft rubber tube (band tubing) 16 inches long, $1\frac{3}{16}$ inches inside diameter and

$1\frac{1}{4}$ inches outside diameter, one end of which is fitted over the mouth of a test tube or suction flask and the other over a $1\frac{5}{8}$ inches key-ring. A clamp completes the outfit (*see* Fig. 19).

Another simple type devised at the Missouri Agricultural Experimental Station by Lasley and McKenzie (Lambert and McKenzie, 1940) consists of an inner tube, $1\frac{5}{16}$ inches in internal diameter and $1\frac{3}{8}$ inches in external diameter, and an outer rubber casing 12 to 15 inches long. An air valve permits adjustment of pressure. The semen is collected in 50 ml. test tubes.

Collection

The rubber tube of McKenzie's simple type of vagina is evenly coated with lubricant, the ring end clamped and the whole apparatus immersed in water at about 45° C. if the apparatus is to be used in cold weather. Water must not be allowed to enter the flask.

A sow is either tied to a wall with a rope round her upper jaw or placed in a simple stanchion. It is usually unnecessary to have a sow on heat. The boar is admitted. When he mounts and attempts to copulate, the open end of the tube is placed in front of his sheath, so that the penis can enter the tube. The tube is manipulated with a pulsating motion of the operator's hand, thus encouraging continued copulation and ejaculation. Too much pressure should not be applied against the sheath, else part of the contents of the preputial diverticulum may be forced into the collecting flask. The semen runs down the rubber tube and is collected in the flask and the clamp is placed on the tube just above the flask. The operations require from 5 to 20 minutes, depending on the condition and disposition of the boar.

The Missouri model requires neither water nor heat and is satisfactory in operation. The correct pressure is adjusted through the air valve prior to use. Pulsations on the penis are made by frequent squeezing of the casing. Otherwise, this apparatus is lubricated and handled as other models.

Insemination

Since spermatozoa are not uniformly ejaculated throughout the period of copulation, the ejaculate should be well mixed before insemination and the gelatinous lumps discarded. These lumps can be separated in a glass bowl by a glass rod. The amount of semen injected is about 50 to 100 ml. and it should be placed well forward in the vagina. The greatest concentration of spermatozoa occurs at certain intervals when the rate of discharge of semen is greatest—usually at the 2nd to 4th minute of ejaculation (McKenzie, Miller, and Bauguess, 1938). If this fraction is used for insemination a smaller volume, of only about 25 ml., may be used after dilution. After introducing the semen a moistened cotton wool plug should be placed in the vestibule of the vagina to prevent waste of semen.

The sow to be inseminated should be secured as for collection of semen. She should be on heat and the optimum time to breed is late on the first day or on the second day of heat.

A 50 ml. syringe with glass barrel, a piece of rubber pressure tubing 45 cm. long and about 4 mm. inside diameter (an ebonite nozzle may be used instead of the rubber tubing) are used for insemination. When the sow is ready the nozzle is introduced into the vagina and forward into the cervix and the semen is slowly expelled.

Dog

The following account is taken from Lambert and McKenzie (1940). Usually semen can be collected easily by manual manipulation. The base of the penis is manipulated with the hand until erection occurs. The prepuce is then pushed behind the bulbous part of the penis so that erection is maintained. Under these conditions the accessory glands show irritation and superfunction, ejaculation occurring when the penis is touched. Semen is conveniently collected in a 25 to 30 ml. glass receptacle. If the semen is to be used immediately after collection it may be collected directly into the glass insemination syringe by removing the plunger and keeping the finger over the nozzle to prevent escape of semen. The average volume of semen from medium-sized dogs is about 7 ml. Collections should not be made oftener than once in 2 days, otherwise the spermatozoa count may be reduced. Semen can also be collected with the artificial vagina (Amantea, 1914; Alifanov, 1933-34)

The bitch should be in the proper stage of heat, preferably the 11th to 13th day after beginning to bleed. The cervix is exposed with a speculum (a short test tube of $\frac{1}{2}$ inch inner diameter, the closed end of which has been cut off and the ends well rounded in a flame is suitable). The tip of the catheter is placed in the cervix and the semen slowly injected. After insemination a moist cotton wool plug should be inserted just inside the vulva and the hindquarters should be raised for 10 to 15 minutes to prevent loss of semen.

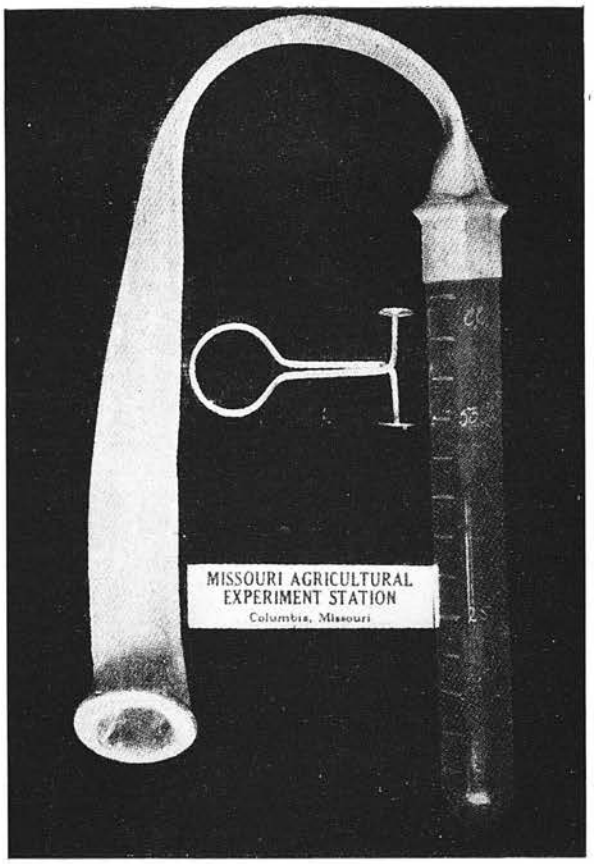


FIG. 19.—Apparatus for the collection of boar semen.

(*From McKenzie*, 1931)

Fowl and Turkey

A method using manual manipulation has been described by Burrows and Quinn (1935, 1937, 1938, 1939) for collecting semen from the cock (*see also* Black and Scorgie, 1942). The bird is held by the thighs by one operator, with its rear towards the second operator, and the legs slightly spread apart so that the abdomen is well exposed. The second operator causes the copulatory organ to protrude slightly from the vent by rapidly massaging the soft part of the abdomen, between the gizzard and the pelvic bones, while the tail of the bird is forced upward over its back with the heel of the left hand. Massage for about 30 seconds generally causes erection and protrusion of the copulatory organ. With continued massage a vigorous orgasm is almost immediately produced and about 0·5 to 1 ml. of thick milky semen ejaculated from the bulbous duct of the organ. As soon as the copulatory organ is seen protruding from the vent, the vent is grasped by the thumb and forefinger of the left hand to force the copulatory organ outward. By a slow milking manipulation of the copulatory organ the semen is expelled into a receptacle held in the second operator's right hand. This usually produces a further 0·5 ml. of semen. It is very important that the bird be held loosely for a tight grip inhibits the desired reaction.

For repeated collections of semen continued rapid massage of the abdomen causes an ejaculatory response which assists in refilling the ducts. In the turkey it is not necessary to attempt to obtain an ejaculatory response for repeated collections. It is possible to " milk " a bird 2 to 6 times at each operation or as long as semen is obtained. Semen should be collected only once a day. The cock usually produces from 0·2 to 2·0 ml. and the turkey from 0·1 to 0·8 ml. semen. Semen is collected in a small glass funnel to which a rubber cork has been fitted as a grip ; the funnel stem opening is filled with paraffin. There is considerable variation in the response of the male, the best being apparently obtained from males kept in batteries or pens.

Another simple method for collecting semen from the cock has been described by Parker (1939). A glass cup $2\frac{1}{2}$ inches in diameter and 1 inch deep is held over the vent by a clamp over the tail and a No. 14 bronze wire spring across the back ; the cup is held firmly by elastic bands. The bird is allowed to tread pullets and he ejaculates into the cup.

Insemination

The method consists of exposing the oviduct and injecting semen into the uterus (Quinn and Burrows, 1936 ; Burrows and Quinn, 1939). To hold the hen the left hand is placed under her breast, the index finger between the legs, and the thumb and other fingers round the legs. The tips of the forefingers grasp the loose skin of the abdomen, pulling the feathers away from the vent and forcing the abdominal contents into the smallest possible space. This should cause the vent to protrude slightly. The right hand is now placed above the vent with the thumb and forefinger extending downward on each side of the vent and the hen's tail is forced upward over her back by the heel of the right hand. With the hands in this position a sudden pressure between them causes the oviduct to be everted as in normal mating.

The turkey is held with its head between the operator's legs, the bird resting on his legs. Its legs are held in the right hand until the left hand is in position on the abdomen. The process is then similar to that in the hen. Considerable pressure may be required to cause eversion of the oviduct, but eversion is easier, as in the hen, if pressure is applied quickly.

A 1 ml. tuberculin type syringe or a 0·5 ml. all-glass syringe is satisfactory for insemination. When the eversion of the oviduct has exposed the orifice, the syringe is inserted about as far as it will slide easily, which is about two inches. The pressure on the abdomen is fully released but slight pressure is maintained on the syringe, which allows it to follow the retraction of the oviduct. The syringe is about one-third covered when the oviduct is fully retracted. The desired amount of semen is now injected. Freshly collected semen is used for insemination. Insemination with 0·1 ml. semen in the hen and 0·05 ml. semen in the turkey at weekly intervals should result in 80% to 90% fertility of the eggs.

Other Animals

In the rabbit semen is readily obtained with the artificial vagina (Macirone and Walton, 1938 ; Cooksey and McKenzie *in* Lambert and McKenzie, 1940). Collection by electrical stimulation, as in the ram, has proved successful in the fox (*see* Lambert and McKenzie, 1940). Insemination has been used successfully in pigeons and doves (Owen, 1941) and in canaries and finches (Bonadonna, 1939*b*).

CHAPTER 10. MANAGEMENT OF SIRES FOR BREEDING

The maintenance of sires in the best possible breeding condition is a highly essential requirement in breeding programmes, whether these involve natural mating or artificial insemination. It is all the more important when intensive use is made of sires as in co-operative breeding associations using artificial insemination, and particularly when proven sires are used. This subject has been reviewed recently by Lambert and McKenzie (1940) and Milovanov (1939), and the methods used for keeping old bulls in active breeding condition have been given by Dunlop (1941). Most of the points summarised here are discussed more fully elsewhere in this monograph.

Condition.—According to Marshall and Hammond (1943) the best condition for breeding in males is a hard one produced by sufficient exercise to work off a surplus of fat, but favouring retention of nitrogenous substances and vitamins. A rising condition is better for reproductive functions than a falling one. Excessive fatness in breeding males is to be avoided. Fat males may produce a semen of inferior quality and they may be slow or fail at service. Dunlop recommended keeping bulls in poor condition " so poor in fact that we are ashamed to have anyone but our own men see them." If they tend to put on fat the feed is cut until they are reduced to a thin condition. This procedure has been found to increase desire, improve the activity of the sperm, and to result in better conception.

Exercise.—The beneficial effect of regular exercise has been noted by several workers. Great stress is laid by Dunlop on plenty of exercise. Males which receive plenty of exercise will usually produce larger ejaculates containing more sperm of higher activity. The males will also be more active and will be capable of more services in a given period of time.

Feeding.—A properly balanced ration should be fed, containing adequate carbohydrate, protein, minerals, and vitamins. Green food should be available both before and during the breeding season. Dunlop fed fairly liberal amounts of grain during the breeding season, but never to an excess that might result in fat, and all the hay the bulls would eat. Alfalfa, clover, or small grain hay that had been cut and properly cured was preferred, but any kind may be used. Very little silage (maize) was used. The feeding of large amounts of silage is regarded by dairy cattle breeders as detrimental to fertility in the bull (*cf.* p. 36).

Number of Services.—No hard and fast rule can be laid down about the permissible number of services. It varies in different individuals and in different species. Intelligent supervision of breeding sires is essential. If a sire exhibits signs of over-use, as shown by lack of keenness to serve or poor quality of semen, his services should be reduced or the animal rested for a while. Where possible, periodic semen examinations should be made. However, it appears that sires can stand up to a more intensive service regime than was hitherto thought desirable. It seems that regular service stimulates keenness to serve. Marshall and Hammond's statement that it is of great importance that a male should be used regularly and not too much at one time, nor too little at another, is fully supported by work on semen.

For bulls, Kirillov (1933-34) suggested that 2 matings a day is the optimum number. Ejaculation every other day appears to be within the capacity of most adult bulls. Dunlop stressed the very careful supervision of the number of services. No bull 10 years old or over is used oftener than every other day and this only when necessary. Not more than 10 times a month is preferred and this only over a comparatively short breeding period from December 15 to June 1. He observed that older bulls get somewhat slower and perhaps not so sure towards the end of the breeding season. For rams it appears that more than 3 matings a day can be made without serious depletion of sperm (*cf.* pp. 45-47).

In the stallion a rest period of 24 hours is regarded as sufficient to restore the number of sperm to normal after frequent matings. It appears that for many mature stallions, 2 to 5 matings may be made on some days without lowering the average level of fertility. But Lambert and McKenzie state that good management would seem to call for lighter use following days with heavy breeding schedules (*cf.* p. 48).

In the boar there should not be more than one mating a day and even then there should be an interval of rest after every 2 days. A yearling boar should probably not be used oftener than every other day if he is to be used for as long as 2 weeks (*cf.* p. 48).

Management.—Males should be docile, well broken to lead, and free from bad habits. Bulls can be kept active and docile to old age by being run in a small paddock and shed with a dry cow for company (Marshall and Hammond). The use of a shield over the eyes often assists the control of a bad-tempered bull and prolongs his breeding life.

Most males will serve best in familiar surroundings. A regular routine should be followed and

the male handled in the same manner each time. The males should be capable of mounting the female promptly on being led out, but better quality semen is said to be produced when the bull is held back for a little before being allowed to mount.

Sires are sometimes upset by travel and on introduction into fresh surroundings. This, however, is usually only temporary, according to Marshall and Hammond, and full fertility can usually be restored by favourable treatment in the way of diet and surroundings. Dunlop advised several weeks of rest after moving an old bull.

It is sound practice to allow males access to cool quarters, an ample supply of cool water for drinking, and ample shade during the summer months in temperate countries. The avoidance of harmful high temperatures is of particular importance in tropical and sub-tropical countries. The age to which male animals are capable of breeding varies with the species and the method of management adopted, according to Marshall and Hammond. This, they state, will depend on the regularity with which services have been distributed throughout the year, the provision of regular and continuous exercise, and the prevention of too great fat accumulation. Dunlop has been successful in the practice of using old proven bulls extensively by paying full attention to condition, exercise, and feeding.

SUPPLEMENT

Chapter 2. The Semen of Animals

The Semen of the Bull

Lasley and Bogart (1943) obtained the following figures for the semen of 12 Hereford bulls in Arizona: volume $4{\cdot}84 \pm 0{\cdot}07$ cm.^{3}, range 0·5-10·5 cm.^{3}; motility rating $4{\cdot}7 \pm 0{\cdot}03$, range 2-6; percentage of live sperm $74{\cdot}22 \pm 0{\cdot}84$, range 30-95; sperm concentration $1{\cdot}16 \pm 0{\cdot}4$ million per mm.^{3}, range 0·1-2·1 million; total sperm per ejaculate—$6{\cdot}11 \pm 0{\cdot}16$ thousand million, range 0·1-18; abnormal sperm $4{\cdot}43 \pm 0{\cdot}21\%$, range 1-18%.

Relation between Different Characteristics (Lasley and Bogart, 1943).—Semen volume was highly correlated with total sperm but not with the other characters. Sperm concentration was significantly positively correlated with total sperm and motility rating, and negatively with the percentage of abnormal sperm. Total sperm per ejaculate was positively correlated with motility rating and percentage of live sperm and negatively with the percentage of abnormal sperm. Motility rating was positively correlated with the percentage of live sperm.

Character of Semen and Fertility.—Swanson and Herman (1944*b*) found that conception rate was not significantly correlated with *p*H, abnormal spermatozoa, or concentration of spermatozoa. A highly significant linear correlation was found between conception rate and viability of sperm on storage. This is more of theoretical than of practical interest because such information is not available until after use of semen, but the correlation can be used as a guide in the development of other methods of semen evaluation; the high correlation of any test with viability should indicate its correlation with semen fertility. A significant curvilinear relationship between conception rate and motility rating at the time of insemination was found, but the difference in conception rate between semen rated 3 motility, which is usually 45% or more progressively motile, and higher grades of motility was slight.

Lasley and Bogart (1943) found that fertility was directly correlated with semen volume, sperm concentration, total sperm per ejaculate (below 16×10^9), and the percentage of sperm resistant to cold shock when diluted with egg-yolk-buffer. Motility rating, percentage of resistant sperm (undiluted), and percentage of abnormal sperm bore no relation to fertility. When live sperm formed less than 50% of the total, fertility was impaired.

Chapter 3. Factors Affecting Semen Production

Temperature.—Asdell and Salisbury (1941) found that spermatogenesis ceased within 24 hours after anchoring the testes of 1-year-old Flemish Giant rabbits in the abdomen. The maximum fertile life of the spermatozoa was 8 days (2 out of 6 animals). Litter size was unaffected. After 9 days none of the 10 animals was fertile. Motile sperm ejaculated 9 days or more after operation did not reach the oviduct.

Chang (1943) caused disintegration of epididymal spermatozoa by application of ice to the scrotal testis. Blocks of ice were applied externally to rabbits' scrota for 10 minutes. After 8-24 hours the tailless sperm in the ejaculates had increased from 0-10% to 24-95%. The occurrence of tailless sperm lasted about 10 days, depending on the rate of evacuation of sperm from the epididymis. Sperm motility was very poor in the 2nd ejaculate 1-2 hours after treatment, indicating that physiological deterioration preceded physical disintegration. There was no effect on the percentage of abnormal sperm (small or large heads), on sperm maturity, on total sperm per ejaculate, or on semen volume. Hence the effect appears to occur on stored sperm in the epididymis and not on spermatogenesis.

Season.—Analyses of variance in various characteristics of the semen production of 3 Beef and 3 Milking Shorthorn bulls studied at intervals of 2 weeks throughout a year revealed significant differences between bulls within breeds for all the factors studied except volume (Phillips, Knapp, Heemstra and Eaton, 1943). Differences between breeds were significant only in volume and total sperm. Significant or highly significant differences were observed between seasons for number of sperm per mm.^{3}, total sperm, and abnormal heads, necks, middle pieces and tails. Total sperm production was highest in spring and lowest in summer, while the number of abnormal heads and necks

was highest in summer and lowest in winter. The 2 breeds responded differently to the effect of season in only the storage characteristics. The bull-within-breed × season interaction was significant only in semen morphology. These results agree on the whole with those of Erb, Andrews and Hilton (1942). It appears that the decrease noted in certain measures of semen quality during summer months is reflected in decreased breeding efficiency ; the highest percentage of fertile matings (69·6%) occurred in April and the lowest (40·8%) in August.

Lasley and Bogart (1943) found that beef bulls in Arizona gave high quality semen as the season progressed from May to September ; the volume of semen and the percentage of live sperm and of resistant sperm increased slightly during this period, but concentration and percentage of abnormal sperm did not change. Swanson and Herman (1944*c*) made a study of the monthly variation of the initial motility, volume, concentration, useful storage period, *p*H, and morphology of the semen of 13 bulls during a 3-year period. The monthly variations in volume, concentration and percentage of total abnormal spermatozoa were not statistically significant. The *p*H of the semen was significantly lower in the summer than in the winter. Initial motility and useful viability were lower in winter than in spring and summer. They interpreted the results as being largely due to the adverse effect of winter weather on the physical well-being and sexual activity of the aged bulls which supplied most of the semen studied.

Anderson (1944*b*) noted highly significant individual and monthly differences in Kenya for the density and motility of the sperm, the *p*H of the semen and the percentage of ejaculations performed. There appears to be a basic seasonal rhythm in bull semen associated with climatic factors, warmer conditions causing stimulation and vice versa. In general there is a seasonal similarity between semen quality and fertility.

Altitude.—Two Corriedale rams imported from Chile to 3200 m. in Peru were studied by Monge and Martin (1942). Both showed azoospermia at first. In one the process was apparently reversible ; spermatozoa appeared later and *p*H, sperm count, and motility approached optimal values. The other still showed azoospermia after 5 months, but at no time any decrease in sexual drive. Martin and Atkins (1942) stated that the semen of these rams and their offspring showed notable differences from that of rams at sea level. There was a wider range in *p*H, higher average sperm count, appearance of juvenile forms, increased percentage of leucocytes, decreased motility and viability. Only 26% of the semens had optimal biological properties. Since 86% of supposedly infertile ewes became fertile with semen of optimal properties, it is concluded that the reduced fertility of sheep at high altitudes is the result of abnormalities in the semen. From a study of spermatogenesis in the cat and rabbit at high altitudes, a transitory inhibitory action of anoxaemia on spermatogenesis is suggested (Monge and Chanez, 1942).

Frequency of Copulation and Ejaculation (Bull).—Lasley and Bogart (1943) showed that, as the interval between collections increased from one up to ten days, semen volume, total sperm per ejaculate, percentage of abnormal sperm, and fertility increased, sperm concentration increased for five days and then decreased, while motility and percentage of live sperm were not significantly affected.

Sexual Behaviour.—The di-ethyl-amino-ethyl-ether of 2 methoxy-6-allyl-phenol in the form of its hydrochloride (Gravitol, Clavitol, Uterol) elicits a characteristic ejaculatory reflex in the mature male guinea-pig (Barkan, 1942). This characteristic effect is attributed to the previously studied combination of the drug's depressing and stimulating action on the central nervous system.

Chapter 4. Physico-Chemical Properties of Semen

Buffering Capacity.—Bull semen is most highly buffered in the region of the *p*H ranges of 4·0 to 5·0 and 9·5 to 10·0 (Willett and Salisbury, 1942). The semen-yolk-phosphate mixture and the semen-yolk-citrate mixture were both highly buffered above *p*H 9·9 and below *p*H 7·5, though the semen-yolk-citrate was more highly buffered below 6·5. In addition to those for bull semen, buffer-coefficient and neutralisation curves are presented for the semen of man, stallion, and dog. From a comparison of these data with buffer-coefficient curves of solutions of citrates, phosphates, and carbonates, the writers conclude that the normal buffer capacity of semen is due primarily to these substances.

Chapter 5. Physiology of Spermatozoa

Metabolism.—Lardy and Phillips (1943*a*) found that malonate, benzoate, fluoride, and hydroquinone reversibly inhibited the respiration and motility of ejaculated bull spermatozoa. Indole, maleate, selenite, arsenite, and quinone inhibited both respiration and motility ; the effects were

irreversible except that inhibition of motility by maleate was reversible in the presence of glucose. Cyanide and azide inhibited respiration, thus indicating the importance of the cytochrome system in the respiration of these cells. Azide depressed motility, the effect being more marked in the presence of glucose. Malonate, cyanide, and benzoate inhibited motility in the absence of glucose, but were relatively non-toxic in the presence of glucose. This is regarded as further evidence of the ability of 2 separate metabolic processes, *i.e.* oxidative and glycolytic, to furnish energy for the motility of bull spermatozoa. Lardy and Phillips (1943*b*) found that malonate and benzoate at concentrations of 0·01M did not appreciably inhibit glycolysis while cyanide, maleate, and hydroquinone stimulated it. The effect of cyanide was reversible ; that of maleate and hydroquinone was not. Fluoride (0·01M) completely and reversibly inhibited glycolysis. Pyruvate reversed fluoride inhibition of glycolysis but was ineffective in relieving fluoride inhibition of motility. In yolk-buffer some specimens of bull spermatozoa could be almost completely inactivated by fluoride for several days and upon transfer to fresh yolk-buffer vigorous motility was regained. Quinone and azide reversibly inhibited glycolysis. Inhibition by iodoacetate or by *dl*-glyceraldehyde was irreversible.

Lardy and Phillips (1943*c*) studied the effect of thyroxine and dinitrophenol on sperm metabolism. Dinitrophenol stimulated glycolysis and respiration of ejaculated bull spermatozoa in the presence of glucose, lactate, and pyruvate, but inhibited endogenous respiration. With glucose in the medium there was a lag before stimulation of respiration by dinitrophenol occurred, but no such lag period occurred with lactate or pyruvate. In contrast to its effect on respiration and glycolysis, dinitrophenol inhibited motility, especially in the absence of the above metabolites, with higher concentrations of dinitrophenol, or after prolonged contact with the spermatozoa. Thyroxine in 1 : 75,000 dilution inhibited respiration and stimulated glycolysis.

Lardy and Phillips (1943*d*) found that the optimum *p*H values for the endogenous respiration of bull, cock, and rabbit spermatozoa were 6·9-7·0, 7·25, and 6·8 respectively. At the optimum *p*H for the species, the endogenous respiration of bull, cock, rabbit, and ram spermatozoa was respectively 21, 7, 11, and 22 mm.^{3} $O_2/10^8$ cells/hr. Varying the phosphate concentration of the medium from 0 to 0·03M did not greatly affect the respiration of bovine sperm but glycolysis and motility in the presence of glucose were greatly depressed in the absence of phosphate. Mg^{++} and K^+ were essential for maintenance of optimum motility, respiration and glycolysis. Mn^{++} and especially Ca^{++} affected motility, glycolysis, and respiration adversely.

Acetate increases the respiration and maintains the motility of epididymal and of 2,4-dinitrophenol treated ejaculated spermatozoa of the bull (Lardy and Phillips, 1944).

Morphology.—Fresh, unstained and unfixed samples of sperm from many fertile bulls studied under the electron microscope have shown that the anterior portion of the sperm head is always enveloped by a protoplasmic cap which appears damaged or disappears altogether if sperm are stained or fixed (Baylor, Nalbandov and Clark, 1943). This suggests that, contrary to the results obtained with the optical microscope, the protoplasmic cap is not a sign of immature or abnormal sperm but is typical of normal sperm when these are examined without being exposed to solvents usually present in stains. The tail ends in a brush consisting of many free and very long filaments. Breaks in the main or end pieces of the tail have also shown flared brushes which make it seem likely that the axial filament consists of a bundle of fine fibres rather than a single relatively thick thread.

Fertility.—Fekete and Duran-Reynals (1943) observed that materials rich in hyaluronidase (mouse and rat testicular extract, rattle-snake venom, leech extract, and purified preparations from sheep testes) diluted in Locke's solution had a pronounced effect in dispersing the follicular cells surrounding the ova of mice. They suggest that hyaluronidase is responsible for this effect (*cf.* p 59).

Chapter 6. Storage and Transport of Semen

Bull Semen.—Beck and Salisbury (1943) investigated rapid methods for estimating the quality of bull semen. The decrease in motility of sperm in bull semen diluted with yolk citrate and stored 10 days at 5° C. after gradual cooling was positively and significantly correlated with the decrease in motility of similar samples stored in water baths for 1 hour at 46·5° C., for 45 minutes at 47° C., and for 30 minutes at 47° C.

A test based on the rate at which semen diluted with yolk-citrate will reduce a dilute solution of methylene blue was suggested for use by operators of artificial breeding circuits. The test is largely dependent on the concentration and motility of sperm and the concentration of ascorbic acid in the semen. These 2 tests may be combined for semen samples and thus information may be rapidly obtained on initial motility, duration of motility, and relative metabolic rate. Sørensen (1941) used

methylene blue as an indication of the dehydrogenating activity of the sperm ; the quicker the bleaching of the methylene blue, the greater the content of motile sperm in the semen. Gunsalus, Campbell, Beck and Salisbury (1944) found that when hygienic precautions are observed, the number of bacteria found in freshly collected semen or freshly prepared yolk-citrate diluent is not sufficient to interfere with the methylene blue reaction for semen quality. The short-time-high-temperature incubation test for semen quality may kill up to 50% of the bacteria present when the test is run at 45° C. or above, especially in the presence of methylene blue. A temperature of 46·5° C. is recommended. The methylene blue reduction test is not suitable as a criterion of the quality of semen stored more than 2 days. The short-time-high-temperature test is recommended as a criterion of continued viability of the spermatozoa in stored semen samples.

Margolin (1943) obtained a highly significant negative correlation between the longevity of semen samples stored in egg-yolk phosphate at 45° F. and the *p*H drop (" acid resistance," determined by titrating semen with 0·1N sulphuric acid until sperm motility reached zero ; the *p*H was determined before and after titration). This acid resistance test requires 5-10 minutes to perform and it is thus possible to estimate semen longevity before semen is shipped or stored.

Lasley and Mayer (1944) have shown that the survival of bull spermatozoa is dependent primarily on a variable physiological factor which is responsible for, or influences, their resistance to adverse environmental conditions. A marked difference was found both in survival during storage and in the resistance to a low temperature shock (10 minutes at 0° C.) between sperm collected from bulls in Arizona and Missouri ; the difference was largely eliminated by the addition of egg-yolk phosphate.

A relationship exists between the survival time of bull sperm under storage conditions and the degree of their resistance to a low temperature shock. The cold shock technique can be used as a measure of the storage potentialities of semen specimens.

The results suggest that the storage potentialities of undiluted semen depend on the number of resistant sperm present at the time of ejaculation, while those of semen diluted with egg yolk are dependent on the number of sperm, in addition to those resistant at ejaculation, possessing the ability to become resistant in the presence of egg yolk. Epididymal sperm were much more resistant to cold shock than ejaculated sperm. A varying proportion of epididymal sperm apparently lose their resistance at the time they are ejaculated, but some of the ejaculated sperm still possess the faculty of becoming resistant.

Swanson and Herman (1944*a*) adapted a small insulated ice cream container for the shipment of bull semen. The semen is placed in an insulated rubber-wrapped vial in a vacuum bottle of water at 40° F. The vacuum bottle is packed in a can of chipped ice and placed in the insulated container. The size of the bottle and the amount of ice used can be varied for different outside temperatures. Semen in transit three days in mid-summer was maintained in good condition and proved satisfactory in settling cows.

Willett and Salisbury (1942) have investigated the effect of dilutor, cooling rate, temperature of storage, and some other factors on the viability of spermatozoa in stored samples of bull semen. In cold weather semen should be collected by the new-type artificial vagina which prevents chilling of the spermatozoa. The writers are inclined to recommend yolk-citrate for routine use in artificial breeding circuits for the following reasons : (1) it maintains motility at a higher level for extended periods of storage than does yolk-phosphate, (2) it disperses fat globules and other materials in the egg yolk so that individual spermatozoa may be seen, and (3) it is easier to prepare because it requires but one chemical.

Their investigations concerning the optimum conditions for storage show that temperature is extremely important, the optimum for long storage being between 1° and 5° C. The optimum rate of cooling for storage at 1° C. was 5 degrees per 2 hours ; for storage at 5° C. the optimum rate was 5 degrees per 20 minutes. They believe that 5° C. will give the best results in practice.

It was consistently found that greater motility was maintained when the semen was covered with a layer of mineral oil than when oil was not used. There was a greater decrease in *p*H when the semen was covered with oil during storage.

Their results indicate that spermatozoa are probably not injured when semen is removed from storage and immediately inseminated into the cow, as is the common practice.

Boar Semen.—Lasley and Bogart (1944) studied some factors affecting the resistance of ejaculated and epididymal spermatozoa of the boar to different environmental conditions. Secretions from the accessory glands did not greatly influence the resistance to cold shock or storage potentialities of boar epididymal spermatozoa. The degree of dilution using several different dilutors had no influence on the resistance of epididymal spermatozoa to cold shock. Fluids from the epididymis obtained by

centrifuging boar epididymal suspension did not influence the resistance to cold shock or the storage potentialities of ejaculated boar spermatozoa.

Dilution of suspensions of epididymal and ejaculated boar spermatozoa with egg-yolk-phosphate buffer increased their resistance to cold shock and their survival under storage conditions.

The resistance of spermatozoa to cold shock varies with the place in the reproductive tract from which they are obtained. Spermatozoa from the head of the epididymis are very resistant and survive during storage for long periods but their resistance and survival capacity decrease as the distance of their location from the testis increases, until spermatozoa in the ejaculate have practically no resistance or storage potentialities. It is suggested that the reduction in these respects is associated with changes within the spermatozoa rather than with changes in their environment.

Chapter 7. The Examination of Semen

Enumeration of Spermatozoa.—Shaffner and Andrews (1943) have described a method for determining the volume of sperm cells in bull and fowl semen by centrifugation.

Staining Method.—Blom (1943) dilutes bull semen with 1% NaCl so that the mixture contains about 200,000 sperm per mm^3. The smear is dried in air and fixed by alcohol or heat. Any NaCl in the preparation is removed by washing with distilled water. The staining solution consists of 1 part sodium carbonate with 9 parts 1% methyl violet solution. The stain, which must be prepared immediately before use, is poured on to the still moist preparation and left for 5 minutes. It is then poured off, the slide washed vigorously with distilled water, blotted and dried in air.

Chapter 8. The Artificial Insemination of Animals

Artificial Insemination of Cattle

Artificial Insemination Associations.—Bartlett and Perry (1944) devised a rental plan based on performance for selected proved bulls and pedigree performance for unproved bulls acceptable to the committee of the association. All charges are on the basis of each acceptable semen sample collected by the technician of the breeding unit.

Use in Different Countries: U.S.A.—Dowell and Winters (1942) have discussed the economic aspects of artificial insemination of commercial dairy cows as revealed by facts and estimates produced by specialists in the 48 states. Artificial insemination was first used commercially in 1938, in which year there were 7 associations in 6 states. By the end of 1941 there were 88 associations in 25 states and the number of cows had increased to 105,126, or about 0·4% of the total dairy cows 2 years old and over. Over three-quarters of the associations charged $5 membership fees and $5 per cow for 3 inseminations. On the average 1·79 inseminations per cow were needed compared with 1·74 for natural service. In comparison $9·50 is estimated as the average cost per service of a bull kept in a herd of 10 cows (the average number per herd served by associations). The average number of cows per association in 1941 was 1195. The practical minimum was estimated at 1000 and the maximum 1500-5000. The maximum distance for effective operation was estimated at 20-30 miles. Therefore, with a local association a concentrated dairy cow population is essential. Developments in Wisconsin and New York indicate the possibilities of large associations capable of dealing with up to 100,000 cows by distributing semen from a central station to sub-stations or to local associations of down to 300 cows. Other factors which will influence future developments are the availability of proved sires and the attitude of farmers towards herd improvement. The average number of cows served per bull has increased from 160 in 1938 to 239 in 1941. In 1941 the average number of bulls per association was 5; the typical 1200-cow association would need to keep 3 proved and 6 unproved sires. The estimated reduction in the number of bulls required compared with natural service was 80-90%. Other economic consequences of an expanded artificial insemination programme include the adoption of improved feeding and management practices, improvement in the health, fertility and genetic make-up of dairy herds, and regional specialisation in the raising of dairy heifers for replacement.

Conception Rate and Factors Influencing It.—Rowson (1944) has reported the results obtained by the Cambridge and District Cattle Breeders' Society; the conception rate for 5 bulls varied from 1·31 to 1·86.

Elliott, Salisbury and Brownell (1943) have investigated some factors influencing success in field artificial insemination. In New York, results are measured by the percentage of cows bred which do not return for further service. A considerable proportion of cows which do eventually

return have not yet done so 1-2 months after service. Records of one association indicate that 99% have returned at the end of 5 months, however. Periods of low fertility appear to occur in mid-winter and spring which is not in agreement with most of the published work on the subject. In fact, one period of relatively high fertility falls in August and September, though most workers have found this to be a period of low fertility. Seasonal variation in semen quality may be less striking in central New York than in some States further south, which may account for part of this deviation from the usual seasonal variation in fertility.

Selection of bulls from a fertility standpoint and the careful selection of semen samples are believed to be factors of primary importance in the maintenance of a good level of fertility. Over 35% of bulls selected for use in one large circuit have been discarded due to unsatisfactory fertility, although their previous breeding history and semen were carefully checked before the bulls were used. The bulls culled were all over 5 years of age and the average age was 8 years.

When it is employed with other routine laboratory measures the methylene blue reduction test assists the technician considerably in the selection of semen samples for use. Semen has been diluted as much as 1 part semen to 16 parts of yolk-citrate dilutor, which increases the use of outstanding bulls of high fertility.

Time of Insemination.—Trimberger and Davis (1943) investigated the conception rate in dairy cattle by artificial insemination at different stages of oestrus. Their results are : start of oestrus, 44·0% ; middle of oestrus, 82·5% ; middle of oestrus and rebred in 24 hours, 84·0% ; end of oestrus, 75% ; 6, 12, 18, 24, 36, and 48 hours after the end of oestrus, 62·5, 32·0, 28·0, 12·0, 8·0 and 0% respectively.

Age of Cows.—Fertility increased with age up to 5-6 years and then fell (Lasley and Bogart, 1943).

Site of Injection.—Intra-uterine insemination was more efficient than intra-cervical and the latter much more efficient than intra-vaginal (Lasley and Bogart, 1943). Very successful results are being obtained by intra-uterine insemination in the United States and at Cambridge (Rowson, 1944).

Artificial Insemination of Sheep

Factors Affecting Insemination Results.—Quinlan, Steyn and de Vos (1941) conducted some experiments on artificial insemination of 196 Merino sheep with fresh and stored semen. Good results were obtained with fresh semen but there was a marked fall in the resulting pregnancies with semen stored longer than 12 hours. In field observations using a Karakul ram to inseminate Afrikaner ewes, 482 ewes suckling their lambs required a total of 763 inseminations for 319 conceptions (2·39 inseminations per conception), *i.e.* 66·18% fertility. 198 ewes without lambs required 372 inseminations for 51 conceptions (3·88 inseminations per conception) *i.e.* 25·76% fertility. The failure to breed in the latter group was considered to be due to the fat condition of the sheep which had not had the constitutional drain of milk production.

Chapter 9. The Technique of Artificial Insemination

Cattle.—At Cambridge (Rowson, 1944) intra-uterine insemination is practised, the semen being deposited at the bifurcation of the uterine horns. A straight glass capillary tube, holding a little over 1 ml. of fluid when full, is used, the end being drawn out and tapered. A 2 ml. glass syringe is attached to the tube by means of a short piece of rubber tubing. A new pipette is used for each animal. The pipettes can be sterilised and there is no chance of spreading infection by means of the inseminating instruments.

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